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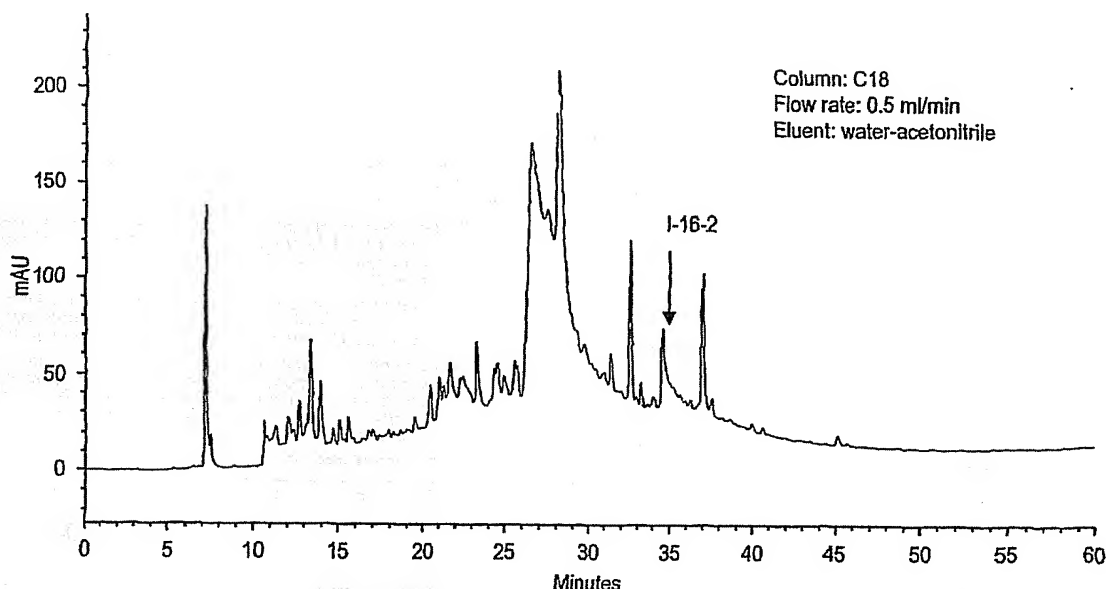
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22 April 2004

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: BOTANICAL EXTRACT COMPOSITIONS WITH ANTI-CANCER OR PHYTOESTROGENIC ACTIVITY COM-
PRISING WOGONIN, ISOLIQUIRITIGENIN AND/OR COUMESTROL



(57) Abstract: A composition having phytoestrogenic and anti-cancer activity is described. The composition comprises wogonin, isoliquiritigenin, coumestrol, their pharmaceutically acceptable salts or esters, their selectively substituted analogs, or combinations thereof. The compositions may also include an anti-cancer agent and/or an immune stimulant. A method for treating or preventing cancer or an estrogen related disorder includes administering a therapeutically effective amount of the compositions is described. The compositions are particularly useful in the treatment of hormone-related cancers.

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INTERNATIONAL SEARCH REPORT

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PCT/US 03/06979

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/352 A61K31/122 A61K31/37 A61K35/78 A61K45/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, WPI Data, PAJ, EMBASE, SCISEARCH, CANCERLIT, MEDLINE, CHEM
ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IKEMOTO SHINICHI ET AL: "Antitumor effects of Scutellariae radix and its components baicalein, baicalin, and wogonin on bladder cancer cell lines" UROLOGY, vol. 55, no. 6, June 2000 (2000-06), pages 951-955, XP002257001 ISSN: 0090-4295 abstract page 951, column 2, paragraph 1 -page 952, column 1, paragraph 4 page 952, column 2, paragraph 5 page 953, column 2, paragraph 2 table 1 --- -/--	1-3,5,6

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

27 February 2004

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Name and mailing address of the ISA

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International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 30342 A (CHEN YANG LING LING ; TRUSTEES OF SOUTHERN ILLINOIS (US); LEE TONY) 3 May 2001 (2001-05-03) page 1, line 15 - line 22 page 14, line 17 -page 16, line 22 page 27, line 3 - line 7 page 29, line 24 - line 30 page 30, line 29 - line 31 example 12 claims 1,2,6,7,11,12 ---	1-3,6
X A	EP 0 742 012 A (KUREHA CHEMICAL IND CO LTD) 13 November 1996 (1996-11-13) page 3, line 57 -page 4, line 5 page 5, line 27 -page 6, line 21 page 11, line 51 - line 56 page 12, line 28 -page 13, line 3 example 4 ---	1-4,6, 31-34 5
X A	LEE WOAN-ROUH ET AL: "Wogonin and fisetin induce apoptosis in human promyeloleukemic cells, accompanied by a decrease of reactive oxygen species, and activation of caspase 3 and Ca ²⁺ -dependent endonuclease" BIOCHEMICAL PHARMACOLOGY, vol. 63, no. 2, 15 January 2002 (2002-01-15), pages 225-236, XP001162761 ISSN: 0006-2952 abstract page 228, column 1, paragraph 1 page 235, column 1, paragraph 3 ---	1-4 5,6
X A X	DATABASE WPI Week 199737, 8 July 1997 (1997-07-08) Derwent Publications Ltd., London, GB; AN 1997-399429 XP002257002 MORINO MASAYOSHI ET AL.: "Flavonoid-containing agent for suppressing synthesis of protein of HSP27 family" & JP 09 176011 A (KUREHA CHEM IND CO LTD), 8 July 1997 (1997-07-08) abstract ---	1-3 5,6
X	WO 00 03706 A (DARRO FRANCIS ;KISS ROBERT (BE); LAFON LABOR (FR); FRYDMAN ARMAND) 27 January 2000 (2000-01-27) page 2, line 23 -page 4, line 9 page 4, line 26 -page 7, line 6 page 14, line 15 -page 39, line 15 claims 1-9 --- -/--	1-4,6, 17,18, 21,22

INTERNATIONAL SEARCH REPORT

International Application No.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 98 48790 A (ANTICANCER INC) 5 November 1998 (1998-11-05) page 1, line 5 - line 9 page 4, line 17 -page 5, line 27 page 9, line 25 - line 26 page 12, line 15 -page 13, line 5 example 2 page 18, line 16 -page 19, line 2 claims 1-3,8,9 ---	1-4 6
X A	EP 0 906 761 A (ARCHER DANIELS MIDLAND CO) 7 April 1999 (1999-04-07) page 3, line 28 - line 32 page 4, line 1 - line 14 page 6, line 32 - line 35 claims 1-6,8,14 ---	1-4, 31-33 5,6
P,X	WO 02 34073 A (FUERST PETER) 2 May 2002 (2002-05-02) page 1, line 1 - line 4 page 3, line 27 -page 4, line 11 page 4, line 24 - line 27 page 5, line 7 - line 16 page 6, line 4 -page 8, line 10 tables 1,2 claims 1-5,7,9,12 ---	1-4,8,9, 17,21
P,X	MIAO-JING LAI ET AL.: "Relative flavone bioavailability of Scutellariae Radix between traditional decoction and commercial powder preparation in humans" J. FOOD AND DRUG ANALYSIS, vol. 10, no. 2, June 2002 (2002-06), pages 75-80, XP001170316 page 75, column 1, paragraph 1 page 77, column 2, paragraph 1 ---	1,3,5,6
X A	WO 98 09615 A (CHEN SOPHIE) 12 March 1998 (1998-03-12) --- page 2, paragraph 4 - paragraph 5 page 4, paragraph 1 -page 5, paragraph 4 example 1 claims 1,7,9 --- -/--	1-3,5,8, 10,17, 19-21, 23,27,28 24

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 01 66123 A (LIU SHWU HUEY ;UNIV YALE (US); CHENG YUNG CHI (US)) 13 September 2001 (2001-09-13) page 1, line 11 - line 13 page 9, line 16 - line 23 table I page 11, line 15 -page 12, line 27 page 20, line 20 - line 26 table 4 page 47, line 13 - line 21 claims 1-3,29,34</p> <p style="text-align: center;">---</p>	<p>1,2,5,8, 10,17, 21,23</p>
X	<p>DATABASE WPI Week 199047 Derwent Publications Ltd., London, GB; AN 1990-352780 XP002268303 IKEGAWA TETSUDO ET AL.: "Carcinostatic assistant" & JP 02 255622 A (TSUMURA & CO), 16 October 1990 (1990-10-16) abstract</p> <p style="text-align: center;">---</p>	<p>1,5,17, 18,21,22</p>
X	<p>WO 01 78783 A (HAUSER INC ;UNIV TEXAS (US)) 25 October 2001 (2001-10-25) page 4, line 5 - line 8 page 12, line 24 -page 13, line 16 tables 1,2 claims 1,22-25,50-52,73-75</p> <p style="text-align: center;">---</p>	<p>1,2,4, 17-22, 27,28</p>
X	<p>WO 95 20960 A (DANA FARBER CANCER INST INC ;ARCH DEV CORP (US); KUFE DONALD (US);) 10 August 1995 (1995-08-10) page 6, paragraph 5 -page 7, paragraph 3 page 9, paragraph 1 page 10, paragraph 4 claims 15-17</p> <p style="text-align: center;">---</p>	<p>1-4,17, 18,21,22</p>
X	<p>DATABASE WPI Week 199429, 21 June 1994 (1994-06-21) Derwent Publications Ltd., London, GB; AN 1994-238658 XP002268304 KAMATAKI TETSUYA: "UDP-glucuronyl transferase inhibitor" & JP 06 172195 A (TSUMURA & CO.), 21 June 1994 (1994-06-21) abstract</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	<p>1,4,17, 18,21,22</p>

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 03707 A (DARRO FRANCIS ;KISS ROBERT (BE); LAFON LABOR (FR); FRYDMAN ARMAND) 27 January 2000 (2000-01-27) page 2, line 22 -page 6, line 11 page 14, line 21 -page 15, line 1 page 15, line 31 -page 16, line 2 page 16, line 11 -page 17, line 11 claims 1-3,7 ---	1-4,6, 17,18, 21,22
X	WO 01 80855 A (CHIN ALLISON C ;GERON CORP (US); GRYAZNOV SERGEI (US); MATRAY TRAC) 1 November 2001 (2001-11-01) page 1, line 9 - line 14 page 9, line 8 -page 10, line 11 page 17, line 16 - line 27 page 18, line 18 -page 20, line 2 page 21, line 8 -page 22, line 2 page 23, line 16 - line 18 ---	1,2,4, 17,18, 21,22, 31-33
X	WO 01 68098 A (BROWN DENNIS M ;CHEMGENEX THERAPEUTICS INC (US); MICHAELS SHAWNYA) 20 September 2001 (2001-09-20) page 8, line 6 - line 13 page 12, line 17 - line 18 page 13, line 2 - line 12 example E036 claims 1,2,6 ---	1,3,4, 17,18, 21,22
E	EP 1 374 880 A (LI HONGFEN) 2 January 2004 (2004-01-02) ---	1-3,5,8, 10,17, 19-21, 27,28
P,X	page 2, line 40 -page 6, line 34 claims ---	
P,X	WO 02 076484 A (AVENTIS PHARMA SA) 3 October 2002 (2002-10-03) page 2, line 4 - line 7 ---	1-4,17, 18,21,22
P,X	US 2003/035851 A1 (CHEN SOPHIE) 20 February 2003 (2003-02-20) ---	1-3,5,8, 10,17, 19-21, 23-25, 27,28 29,30
P,A	page 1, paragraph 3 - paragraph 5 page 1, paragraph 9 page 2, paragraphs 29,30,32 page 4, paragraphs 44-46 page 5, paragraph 50 claims 1,9,10,38-47 ---	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI Week 198543, 12 September 1985 (1985-09-12) Derwent Publications Ltd., London, GB; AN 1985-266688 XP002268305 TAKAHASHI NOBUTAKA ET AL.: "Carcinostatic agent" & JP 60 178815 A (RIKAGAKU KENKYUSHO), 12 September 1985 (1985-09-12) abstract</p>	1,3,4,8, 9,12
X	<p>& PATENT ABSTRACTS OF JAPAN vol. 010, no. 023 (C-325), 29 January 1986 (1986-01-29) & JP 60 178815 A (RIKAGAKU KENKYUSHO), 12 September 1985 (1985-09-12) abstract</p>	1,3,4,8, 9,12
X	<p>--- MA JING ET AL: "Apoptosis induced by isoliquiritigenin in human gastric cancer MGC-803 cells" PLANTA MEDICA, vol. 67, no. 8, November 2001 (2001-11), pages 754-757, XP008026919 ISSN: 0032-0943 the whole document</p>	1,8,12
X	<p>--- EP 0 656 213 A (HYAL PHARMA CORP) 7 June 1995 (1995-06-07)</p> <p>page 12, line 10 - line 31 examples CASEI-VI, CASEXIA, XIB</p>	1,2,8,9, 17, 19-21, 27,28
X	<p>--- FR 2 658 420 A (GAUDEAU CLAUDE; GOUTHIERE LAURENT) 23 August 1991 (1991-08-23) page 1, line 46 - line 56 page 3, line 23 - line 38</p>	1,2,8,10
X	<p>--- DATABSE WPI Week 199409, 1 February 1994 (1994-02-01) Derwent Publications Ltd., London, GB; AN 1994-071834 XP002268306 AOKI YASUO ET AL.: "Enhancer for carcinostatic activity" & JP 06 024975 A (DAINIPPON INK & CHEMICALS), 1 February 1994 (1994-02-01) abstract</p> <p>--- -/--</p>	1,8,12, 17,18, 21,22

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 291 151 A (LEVEEN HARRY H ;LEVEEN ROBERT F (US); LEVEEN ERIC G (US)) 17 November 1988 (1988-11-17) column 1, line 1 - line 12 column 3, line 24 - line 38 claims 1,5-7,12 ---	1,8,9, 17,18, 21,22
X	DATABASE WPI Week 198701, 21 November 1986 (1986-11-21) Derwent Publications Ltd., London, GB; AN 1987-003722 XP002268307 KINOSHITA MASAHIKO: "Carcinostatic adjuvant" & JP 61 263925 A (KANEBO LTD), 21 November 1986 (1986-11-21) abstract ---	1,8,10, 17,18, 21,22
P,X	WO 02 080951 A (SYNERGISTIX BIOTECH INC ;COHEN ISAAC (US)) 17 October 2002 (2002-10-17) page 1, line 5 - line 21 claims 1,5,9 ---	1,2,5,8, 10,13,14
X	WO 01 03716 A (SUN FARM CORP ;SUN ALEXANDER S (US)) 18 January 2001 (2001-01-18) page 3, paragraph 3 page 4, paragraph 1 page 4, paragraphs 5,6 page 7, paragraph 2 - paragraph 3 page 8, paragraph 2 page 10, paragraphs 2,5 page 18, paragraph 2 page 19, paragraph 4 - paragraph 5 claims 1,7,9-11 ---	1-4,7, 13,16, 17, 19-21, 27,28
X	WO 01 21009 A (CURRIER STEPHEN J ;FRIEDMAN ELLIOT P (US); JOHNSTON PAUL D (US)) 29 March 2001 (2001-03-29) page 2, line 3 - line 10 page 5, line 3 - line 5 tables 1B,1C ---	1,3,4,7, 13,15, 16,21
X	EP 1 029 545 A (GREENPOWER INTERNATIONAL NATUU) 23 August 2000 (2000-08-23) page 4, line 26 - line 29 example REZEPTUR12 ---	1,14,17, 18,21-23

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>GB 2 346 325 A (WASSEN INT LTD) 9 August 2000 (2000-08-09) page 1, line 3 - line 4 page 3, line 4 - line 16 page 4, line 26 - line 29 page 5, line 11 - line 16 claims 1,5,6,11,12</p> <p>---</p>	1,2,13, 14
X	<p>KUBO M ET AL: "SCUTELLARIAE RADIX 7. ANTI-ARTHRITIC AND ANTI-INFLAMMATORY ACTIONS OF METHANOLIC EXTRACT AND FLAVONOID COMPONENTS FROM SCUTELLARIAE RADIX" CHEMICAL AND PHARMACEUTICAL BULLETIN (TOKYO), vol. 32, no. 7, 1984, pages 2724-2729, XP008026902 ISSN: 0009-2363 abstract page 2725, paragraph 5 - paragraph 7 table IV page 2728, paragraphs 1,2 page 2728, paragraph 6</p> <p>---</p>	31-34
X	<p>EP 0 633 022 A (KUREHA CHEMICAL IND CO LTD) 11 January 1995 (1995-01-11) page 2, line 5 - line 16 page 2, line 48 -page 3, line 32 table 1 page 6, line 33 - line 47 claims 1-3,7,9</p> <p>---</p>	31-33
X	<p>US 5 935 996 A (YAMAGUCHI MASAYOSHI) 10 August 1999 (1999-08-10) column 1, line 60 -column 2, line 5 example 1</p> <p>---</p>	31-33
X	<p>EP 1 127 572 A (BASF AG) 29 August 2001 (2001-08-29) page 2, line 1 - line 5 page 2, line 27 - line 56 tables 1,4 page 6, line 1 - line 39 page 8, line 10 - line 37 page 12, line 37 - line 41 claims 1,2,4,5</p> <p>---</p> <p style="text-align: center;">-/--</p>	31-34

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI Week 199626, 23 April 1996 (1996-04-23) Derwent Publications Ltd., London, GB; AN 1996-255073 XP002268308 KUMAGAI KAZUO ET AL.: "Inhibitor of matrixmetalloprotease" & JP 08 104628 A (SUMITOMO PHARMA), 23 April 1996 (1996-04-23) abstract</p> <p>---</p>	31-33
X	<p>DATABASE WPI Week 200066, 5 September 2000 (2000-09-05) Derwent Publications Ltd., London, GB; AN 2000-675074 XP002268309 OKADA TADASHI ET AL.: "Oral antibacterial agent" & JP 2000 239136 A (ORIZA YUKA KK), 5 September 2000 (2000-09-05) abstract</p> <p>---</p>	31-33
X	<p>DATABASE WPI Week 199247, 8 October 1992 (1992-10-08) Derwent Publications Ltd., London, GB; AN 1992-385398 XP002268310 MATSUURA MASARU: "Agents for alleviating periodontitis" & JP 04 283518 A (KIKKOMAN CORP), 8 October 1992 (1992-10-08) abstract</p> <p>---</p>	31-33
X	<p>DE 101 18 999 A (REINERS FRITZ) 8 November 2001 (2001-11-08) column 2, paragraph 11 column 3, paragraph 17 claims 1,4</p> <p>---</p>	31-33,35
X	<p>WO 97 11692 A (CAMPBELL R NELSON ;OSTEOARTHRITIS SCIENCES INC (US); SHARPE THOMAS) 3 April 1997 (1997-04-03) page 2, line 18 - line 24 page 5, line 2 - line 7 page 12, line 6 - line 8 page 12, line 32 - line 35 claims 1,2</p> <p>---</p> <p>-/--</p>	31-33

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International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 200145, 24 April 2001 (2001-04-24) Derwent Publications Ltd., London, GB; AN 2001-421066 XP002268311 & JP 2001 114675 A (F) abstract	31-33
P,X	--- WO 02 076241 A (VAN NORREN KLASKE ;NUTRICIA NV (NL); LANSINK MIRIAN (NL); GROS-VAN) 3 October 2002 (2002-10-03) page 3, line 11 - line 22 page 5, line 29 -page 6, line 2 page 6, line 8 - line 14 figure 1 claims 1,2	31-33
A	--- KUTTAN R ET AL: "POTENTIAL ANTICANCER ACTIVITY OF TURMERIC (CURCUMA LONGA)" CANCER LETTERS, NEW YORK, NY, US, vol. 29, no. 2, November 1985 (1985-11), pages 197-202, XP001120343 ISSN: 0304-3835 abstract page 197, paragraph 1 page 201, paragraph 2	26
A	--- DATABASE WPI Week 197803, 3 December 1977 (1977-12-03) Derwent Publications Ltd., London, GB; AN 1978-05666A XP002268312 MATSUI TOKUTAROU: "antitumor agent" & JP 52 145509 A (MATSUI TOKUTAROU), 3 December 1977 (1977-12-03) abstract	25
A	--- GB 1 476 016 A (NIPPON SHINYAKO CO LTD) 10 June 1977 (1977-06-10) the whole document	19,30
A	--- DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1995 OHNO NAOHITO ET AL: "Resistance of highly branched (1 fudarw 3)-beta-D-glucans to formolysis" Database accession no. PREV199598411947 XP002268301 abstract & CHEMICAL AND PHARMACEUTICAL BULLETIN (TOKYO), vol. 43, no. 6, 1995, pages 1057-1060, ISSN: 0009-2363	29,30
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/06979

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-20 and 31-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-2 (partially), 3-6 (entirely)

A method of treating a human in need of cancer treatment, comprising administering a composition comprising greater than 0.5 weight percent based on the total weight of the composition of wogonin, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, or a combination of one or more of the foregoing compounds, wherein the composition does not further comprise isoliquiritigenin and/or coumestrol, and/or an anti-cancer agent, and/or an immune stimulant.

2. Claims: 1-2 (partially), 7 (entirely), 17-30 (partially)

A method of treating a human in need of cancer treatment, comprising administering a composition comprising greater than 0.5 weight percent based on the total weight of the composition of wogonin, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, or a combination of one or more of the foregoing compounds wherein the composition further comprises isoliquiritigenin and/or coumestrol, and/or an anti-cancer agent, and/or an immune stimulant. A composition comprising a combination of 0.5 weight percent based on the total weight of the composition of wogonin and an anticancer-agent, and optionally an immune stimulant.

3. Claims: 1-2 (partially), 8-10 (entirely), 11-12 (entirely), 17-30 (partially)

A method of treating a human in need of cancer treatment, comprising administering a composition comprising greater than 0.5 weight percent based on the total weight of the composition of isoliquiritigenin, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, or a combination of one or more of the foregoing compounds; wherein the composition further optionally comprises wogonin and/or coumestrol, and/or an anti-cancer agent, and/or an immune stimulant (as far as not comprised in the previous subject). A composition comprising a combination of 0.5 weight percent based on the total weight of the composition of isoliquiritigenin and an anticancer-agent, and optionally an immune stimulant.

4. Claims: 1-2 (partially), 13-16 (entirely), 17-30 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method of treating a human in need of cancer treatment, comprising administering a composition comprising greater than 0.5 weight percent based on the total weight of the composition of coumestrol, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, or a combination of one or more of the foregoing compounds; wherein the composition further optionally comprises wogonin and/or isoliquiritigenin, and/or an anti-cancer agent, and/or an immune stimulant (as far as not comprised in the previous subjects). A composition comprising a combination of 0.5 weight percent based on the total weight of the composition of coumestrol and an anticancer-agent, and optionally an immune stimulant.

5. Claims: 31-35 (entirely)

A method of treating a human in need of treatment for an estrogen-related disorder, comprising administering a composition comprising greater than 0.5 weight percent based on the total weight of the composition of wogonin, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, or a combination of one or more of the foregoing compounds, optionally in combination with isoliquiritigenin and/or coumestrol.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-4, 6-9, 11-13, 15-28 and 31-35 relate to compounds which actually are not well-defined. The use of the definitions "their/a pharmaceutically acceptable ester(s)", "their/a selectively substituted analog(s)" and "a glycoside of wogonin" in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. The lack of clarity is such as to render a meaningful complete search impossible.

Consequently, the search has been restricted to the compounds specified in the claims, namely wogonin, genistein, biochanin, prunetin, scutellarein, daidzin, luteolin, apigenin, acacetin, isoliquiritigenin, phloretin and coumestrol, as well as the plant extracts mentioned in claims 5, 10 and 14. No structures or Registry Numbers could be associated with 3,4,6-trihydroxyflavone, 7,3-dihydroxy-4,1-dimethoxy-isoflavone, 3R-2',3'-dihydroxy-7,4-dimethoxy-isoflavone and 4,2,4'-trihydroxychalcone; therefore, these compounds have not been included in the search.

Present claims 17, 19, 21 and 27 relate to compounds defined by reference to a desirable characteristic or property, namely "an anti-cancer agent" or "an immune stimulant". The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to their pharmacological profile. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

In addition, claims 20 and 28 relate to compounds which actually are not well-defined. The use of the definitions "ginsenosides", "glycoproteins", "interferones" and "gamma-globulins" is considered to lead to a lack of clarity within the meaning of Article 6 PCT. The lack of clarity is such as to render a meaningful complete search impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the anticancer agents specifically mentioned in claims 18 and 22-26 and the immune stimulants ferulic acid, mannan, synanthrin, eleutheroside A, B, C, D and E, gynoside, beta-pachyman, inulin, extracts of *Ganoderma lucidum*, extracts of *Coriolus versicolor* or extracts of *Poria cocos* (claims 20 and 28).

Present claims 31-35 relate to a very large number of possible diseases, which have in common that they are estrogen-related disorders. The use of compositions containing > 0.5 wt% of wogonin or salts, esters or analogs thereof for the treatment of menopausal-related symptoms is already known and is considered to be due to their estrogenic activity (see e.g. EP0906761). Therefore, the idea to use a composition comprising greater than 0.5 wt% based on the total weight of the composition of wogonin or salts, esters or analogs thereof for the treatment of estrogen-related

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

disorders cannot fulfill the role of "special technical feature" involved in the technical relationship between the diseases that had been originally regrouped under "invention 5". Only the first invention mentioned in claims 31-35 has therefore been searched, namely, the use of a composition comprising greater than 0.5 wt% based on the total weight of the composition of wogonin, genistein, biochanin, prunetin, scutellarein, daidzin, luteolin, apigenin or acacetin for the treatment of bone loss, bone fractures, osteoporosis, glucocorticoid-induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, rheumatoid arthritis, osteoarthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy and cartilage degeneration.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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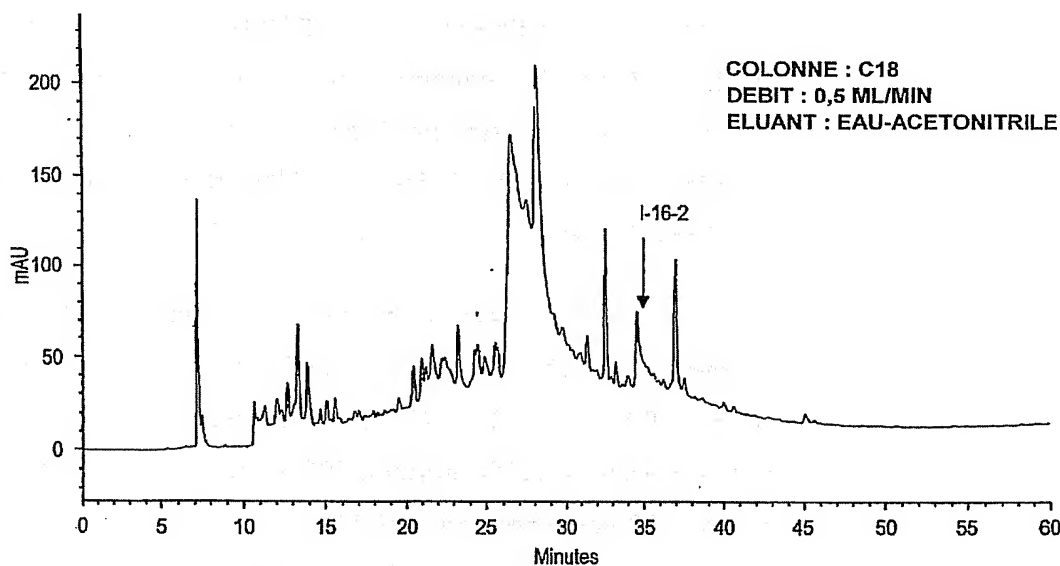
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **BOTANICAL EXTRACT COMPOSITIONS AND METHODS OF USE**



(57) Abstract: A composition having phytoestrogenic and anti-cancer activity is described. The composition comprises wogonin, isoliquiritigenin, coumestrol, their pharmaceutically acceptable salts or esters, their selectively substituted analogs, or combinations thereof. The compositions may also include an anti-cancer agent and/or an immune stimulant. A method for treating or preventing cancer or an estrogen related disorder includes administering a therapeutically effective amount of the compositions is described. The compositions are particularly useful in the treatment of hormone-related cancers.



WO 03/075943 A2

BOTANICAL EXTRACT COMPOSITIONS AND METHODS OF USE

TECHNICAL FIELD

This application related to botanical extract compositions and methods of treating humans, particularly methods of treating cancer and estrogen-related disorders.

BACKGROUND

Botanical extracts Phytoestrogens, such as the isoflavones contained in soy products, are believed to have therapeutic potential in disease treatment and prevention. In particular, phytoestrogens are believed to be useful in the treatment of estrogen-related disorders such as, for example, osteoporosis, the symptoms of menopause, and hormone-related cancers.

It has been reported that endogenous and exogenous hormones play a role in the development of hormone-related cancers, such as breast cancer, colon cancer, lung cancer, endometrial cancer, ovarian cancer, prostate cancer, bladder cancer, testicular cancer, thyroid cancer, and bone cancer (see, for example, Henderson et al, "Hormonal carcinogenesis", *Carcinogenesis* (2000), 21(3): 427-433). Epidemiological studies have shown that consumption of a diet with high content of phytoestrogens such as those found in soy products was associated with a lower incidence of hormonal related cancers (H. Wiseman, "The therapeutic potential of phytoestrogens", *Expert. Opin. Investig. Drugs* (2000), 9(8):1829-40).

Prostate carcinoma, a hormone-related cancer, is a major health problem among men in North America and Europe (S. H. Landis et al., "Cancer Statistics, 1998", *CA Cancer J. Clin.* (1998) 48: 6-29). Chronic enlargement of the prostate in combination with elevated prostate specific antigen (PSA) can often lead to prostate carcinoma. Every year 160,000 new cases and 39,000 deaths from the disease occur in the United States (Landis). Breast cancer, another hormone-related cancer, is also a major health problem. New invasive incidences of breast carcinoma are projected to be 192,200, with 40,200 projected deaths in 2001 according to the American Cancer

Society (National Alliance of Breast Cancer Organizations News, 15(1): 2, January, 2001). Early detection and early intervention are often the key solutions to treating these diseases. Although chemotherapy is often the choice for advanced-stage breast cancer patients, for example, it is not effective for the advanced-stage prostate cancer patients. Conventional treatment methods include surgery, radiation, hormone therapy, and chemotherapy. There is a need for alternative therapeutic agents that can augment or replace existing therapies.

While the existing therapeutic agents are well-suited for their intended purpose, there remains a need for other herbal remedies for the treatment of estrogen-related disorders, including hormone-related cancers, in addition to remedies for non-hormone-related cancers.

BRIEF SUMMARY

Other embodiments, including compositions useful for treating cancer, are described in detail below.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a high performance liquid chromatogram (measured at 254 nanometers) of a multi-component botanical extract composition containing extracts of *Panax pseudo-ginseng* Wall, *Isatis Indigotica* Fort, *Ganoderma lucidium* Karst, *Dendrothema morifolium* Tzvel, *Glycyrrhiza glabra* L, *Scutellaria baicalensis* Georgi, *Rabdosia rubescens*, and *Serenoa repens*; an arrow indicates the position of wogonin (designated "T-16-2") in the elution profile.

Figure 2 shows ^{13}C NMR spectra of wogonin separated from a multi-component botanical extract compositions as in Figure 1; (a) separate (DEPT) spectra for $-\text{CH}_3$, $-\text{CH}_2$ and $-\text{CH}$ groups; (b) total ^{13}C NMR spectrum.

25 Figure 3 is a mass spectrum of wogonin separated from a multi-component botanical extract as in Figure 1, with a purity of greater than 95%.

Figure 4 is a high performance liquid chromatogram of isoliquiritigenin isolated from *Glycyrrhiza uralensis*.

Figure 5 is an absorption spectra associated with the isoliquiritigenin peak in the chromatogram of Figure 4.

5 Figure 6 shows ^{13}C NMR spectra of isoliquiritigenin separated from *Glycyrrhiza uralensis*; (a) separate (DEPT) spectra for $-\text{CH}_3$, $-\text{CH}_2$ and $-\text{CH}$ groups; (b) total ^{13}C NMR spectrum.

Figure 7 is a mass spectrum of isoliquiritigenin separated from *Glycyrrhiza uralensis*., with a purity shown to be higher than 95% .

10 Figure 8 is a plot of cell viability of LNCaP and DU-145 prostate cancer cells as a function of wogonin concentration.

Figure 9 is a plot of cell viability of DU-145 and LNCaP prostate cancer cells, and MCF-7 breast cancer cells, as a function of isoliquiritigenin concentration.

15 Figure 10 displays DNA histograms showing the effect on LNCaP cell cycle in the absence (A) and presence (B) of wogonin at 20 micrograms/milliliter.

Figure 11 shows changes in the LNCaP cell cycle induced by wogonin and isoliquiritigenin.

Figure 12 shows changes in the DU-145 cell cycle induced by wogonin and isoliquiritigenin.

20 Figure 13 is a plot showing the potency of wogonin and isoliquiritigenin as ER-alpha-Luc reporter gene activation.

Figure 14 is a plot showing the potency of wogonin and isoliquiritigenin as ER-beta-Luc reporter gene activation.

25 Figure 15 is a plot of COX-2 inhibition as a function of isoliquiritigenin concentration.

Figure 16 is a plot of cell viability of PTX 10 ovarian cancer cells (resistant to taxol) in the presence of increasing concentrations of wogonin.

Figure 17 is a plot of cell viability of PTX 10 ovarian cancer cells in the presence of increasing concentrations of isoliquiritigenin.

5 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Disclosed herein are compositions and methods for treating cancer and estrogen-related disorders in a human in need of such treatment. As used herein, a human in need of cancer treatment may be a human diagnosed with cancer, or a human wanting to prevent or delay the onset of cancer, for example, a human with a family history of cancer. The cancer may optionally be a hormone-related cancer such as, for example, prostate cancer, breast cancer, endometrial cancer, colon cancer, lung cancer, bladder cancer, testicular cancer, ovarian cancer, thyroid cancer, or bone cancer. As used herein, a human in need of treatment for an estrogen-related disorder may be a human diagnosed with an estrogen-related disorder such as, for example, osteoporosis or the symptoms of menopause, or a human wanting to prevent or delay the onset of an estrogen-related disorder. The method comprises administering a therapeutically effective amount of a composition comprising a phytoestrogen, such as, for example, wogonin, isoliquiritigenin, coumestrol, their pharmaceutically acceptable salts or esters, their selectively substituted analogs, and combinations comprising one or more of the foregoing compounds. As used herein, a phytoestrogen is a plant-derived compound or its metabolite that can mimic the action or modulate the binding, metabolism, or production of endogenous estrogens in the body.

As stated previously, herbal remedies have been used in the treatment of cancer. *Scutellaria baicalensis*, for example, is a source of wogonin (Y. Y. Zhang et al., "Comparative study of *Scutellaria planipes* and *Scutellaria baicalensis*", *Biomed. Chromatogra.* (1998), 12: 31-3), and *Glycyrrhiza uralensis* and *Glycyrrhiza glabra* are sources of isoliquiritigenin (H. Hayashi H. et al., "Seasonal variation of glycyrrhizain and isoliquiritigenin glycosides in the root of *glycyrrhiza glabra* L", *Biol. Pharm. Bull.* (1998) 21: 987-9).

Coumestrol is a phytoestrogen found in alfalfa and red clover that is known to exhibit phytoestrogenic activity (see, for example, U.S. Patent Application Publication No. 20010044431 A1 to Rodriguez).

Wogonin has been reported to be a strong anti-inflammation agent due to its inhibitory activity against cyclooxygenase 2 (COX-2) directly and against gene expression of inducible COX-2 and nitric oxide synthase (see, for example, Y. S. Chi et al., "Effect of wogonin, a plant flavone from *Scutellaria radix*, on the expression of cyclooxygenase-2 and the induction of inducible nitric oxide synthase and the induction of inducible nitric oxide synthase in inhibitors and lipopolysaccharide-treated RAW 264.7 cells", *Biochem. Pharmacol.* (2001), 61(11): 1417-27). However, the present inventor is aware of no reports of wogonin exhibiting estrogenic activity.

Isoliquiritigenin has been reported to possess estrogen-like activity (see, for example, S. Tamir "Estrogen-like activity of glabrene and other constituents isolated from licorice root", *J. Steroid Biochem. Mol. Biol.* (2001), 78(3): 291-8). However, the present inventor is aware of no report that isoliquiritigenin is an inhibitor for COX-2 activity and thus is beneficial to treat cancer.

Recent studies have revealed the importance of COX-2 inhibitors as cancer therapeutic agents (see, for example, A. Kirschenbaum et al., "The role of cyclooxygenase-2 in prostate cancer" *Urology* (2001), 58(2 suppl. 1): 127-131; and E. T. Hawk et al., "COX-2 in Cancer-A Player That's Defining the Rules" *J. Natl. Cancer Inst.* (2002), 94(8): 545-546). The inhibitor blocks the angiogenesis of cancer and reduced the cancer metastasis (see, for example, E. Fosslien "Review: Molecular pathology of cyclooxygenase-2 in cancer-induced angiogenesis", *Ann. Clin. Lab. Sci.* (2001), 31(4): 325-348).

The present work demonstrates potent activity of wogonin and isoliquiritigenin to activate estrogen receptor-alpha and -beta and trigger biochemical reactions in cancer cells. The COX-2 inhibitory activity of isoliquiritigenin is also demonstrated. Suppressive effects of both compounds on cancer cell proliferation are also demonstrated. The cytotoxicity of wogonin and isoliquiritigenin toward cancer cells may either be dependent or independent of estrogen receptors.

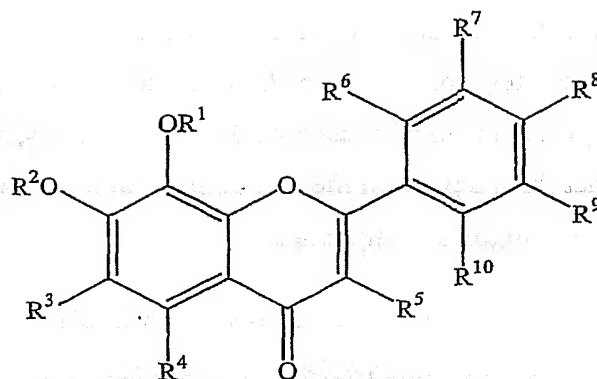
The phytoestrogen may comprise wogonin. As used herein, the term wogonin encompasses CAS Reg. No. 632-85-9, also known as 5,7-dihydroxy-8-methoxy-flavone, and its pharmaceutically acceptable salts or esters, its selectively substituted analogs, an extract from a plant of the *Scutellaria* family, or a combination comprising one or more of the foregoing compounds.

An ester of wogonin is preferably a glycoside of wogonin. There is no particular limit on the monosaccharide or polysaccharide used to form the glycoside of wogonin. Suitable monosaccharides sugars include, for example, glucose, glucuronic acid, mannose, fructose, galactose, xylose, rutinose, rhamnose, and the like, and combinations comprising one or more of the foregoing monosaccharides. Suitable polysaccharides include, for example, dimers, trimers, oligomers, and polymers formed from one or more of the above monosaccharides.

Wogonin analogs include, for example, genistein, biochanin, prunetin, scutellarein, daidzin, luteolin, apigenin, acacetin, 3,6,4-trihydroxyflavone, 7,3-dihydroxy-4,1-dimethoxy-isoflavone, 3R-2',3'-dihydroxy-7,4-dimethoxy-isoflavone, or the like, or a combination comprising one or more of the foregoing wogonin analogs.

Wogonin can also be in the form of an extract from a plant of the *Scutellaria* family such as, for example *Scutellaria baicalensis*, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, or a combination comprising one or more of the foregoing compounds.

The wogonin can comprise a selectively substituted analog of wogonin having the formula



wherein R^1 is C_1 - C_6 alkyl (preferably methyl); R^2 is hydrogen, C_1 - C_6 alkyl, or C_2 - C_6 acyl (preferably hydrogen); and R^3 - R^{10} are independently hydrogen, hydroxy, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, or C_2 - C_6 acyl (preferably hydrogen or hydroxy, more preferably hydrogen), with the proviso that at least four of R^3 - R^{10} are hydrogen. In a preferred embodiment, R^1 is methyl, R^2 is hydrogen, and R^3 - R^{10} are independently hydrogen, methyl, ethyl, methoxy, ethoxy, acetyl, or propionyl, with the proviso that at least five of R^3 - R^{10} are hydrogen.

Methods for synthesizing or isolating wogonin, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, are known in the art. See, for example, International Patent Application No. WO01051482 A1 to Wallace et al; P. Rivaille et al., *C. R. Acad. Sci., Paris, Ser. C* (1969), 268(2): 2213-16; M. -C. Lin et al., *J. Chromatogr. A* (1999), 830(2): 387-395; Y. -C. Chen et al., *Biochem. Pharmacol.* (2001), 61(11): 1417-1427.

When wogonin is present, the wogonin comprises greater than or equal to 0.5 weight percent, more preferably greater than or equal to about 1 weight percent, still more preferably greater than or equal to about 2 weight percent, even more preferably greater than or equal to about 5 weight percent, even more preferably greater than or equal to about 10 weight percent, still more preferably greater than or equal to about 20 weight percent of the total weight of the composition. Compositions containing as much as 50 weight percent, or even as much as 100 weight percent of wogonin are contemplated.

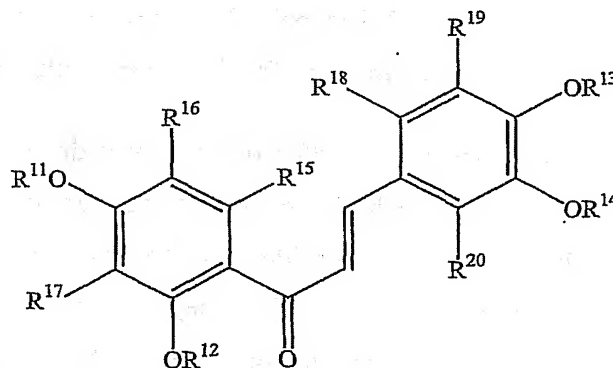
The phytoestrogen may comprise isoliquiritigenin. As used herein, isoliquiritigenin refers to CAS Reg. No. 961-29-5; also known as 2',4,4'-trihydroxychalcone, a pharmaceutically acceptable salt or ester of isoliquiritigenin, a selectively substituted analog of isoliquiritigenin, an extract of *Glycyrrhiza uralensis* or *Glycyrrhiza glabra*, or a combination comprising one or more of the foregoing compounds.

An ester of isoliquiritigenin is preferably a glycoside of isoliquiritigenin. There is no particular limit on the monosaccharide or polysaccharide used to form the glycoside of isoliquiritigenin. Suitable monosaccharides sugars include, for example, glucose, glucuronic acid, mannose, fructose, galactose, xylose, rutinose, rhamnose, and the like, and combinations comprising one or more of the foregoing monosaccharides. Suitable polysaccharides include, for example, dimers, trimers, oligomers, and polymers formed from one or more of the above monosaccharides.

An isoliquiritigenin analog includes, for example, phloretin, 4,2,4'-trihydroxychalcone, or the like, or a combination comprising one or more of the foregoing isoliquiritigenin analogs.

An extract of *Glycyrrhiza uralensis* or *Glycyrrhiza glabra* is a source of isoliquiritigenin, a pharmaceutically acceptable salt or ester of isoliquiritigenin, a selectively substituted analog of isoliquiritigenin, or a combination comprising one or more of the foregoing compounds.

A selectively substituted analog of isoliquiritigenin has the formula



wherein R^{11} - R^{14} are independently hydrogen or C_1 - C_6 alkyl (preferably hydrogen); and R^{15} - R^{20} are independently hydrogen, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, or C_2 - C_6 acyl (preferably hydrogen), with the proviso that at least three of R^{15} - R^{20} are hydrogen. In a preferred embodiment, R^{11} - R^{14} are hydrogen and R^{15} - R^{20} are independently
5 hydrogen, methyl, ethyl, methoxy, ethoxy, acetyl, or propionyl, with the proviso that at least four of R^{15} - R^{20} are hydrogen.

Methods for synthesizing or isolating isoliquiritigenin, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, are known in the art. See, for example, S. K. Srivastava et al., *Indian J. Chem., Sect. B* (1981), 20B(4): 347-8;
10 and F. A. Macias et al., *Phytochemistry* (1998), 50(1): 35-46.

When isoliquiritigenin is present, the isoliquiritigenin comprises greater than or equal to 0.5 weight percent, more preferably greater than or equal to about 1 weight percent, still more preferably greater than or equal to about 2 weight percent, even more preferably greater than or equal to about 5 weight percent, even more preferably
15 greater than or equal to about 10 weight percent, still more preferably greater than or equal to about 20 weight percent of the total weight of the composition. Compositions containing as much as 50 weight percent, or even as much as 100 weight percent of isoliquiritigenin contemplated.

The phytoestrogen may comprise coumestrol. As used herein, coumestrol
20 refers to CAS Reg. No. 479-13-0, also known as 3,9-dihydroxy-6H-benzofuro[3,2-c][1]benzopyran-6-one, a pharmaceutically acceptable salt or ester of coumestrol, a selectively substituted analog of coumestrol, an extract of *Taraxacum mongolicum*, alfalfa sprout (*Medicago sativa*), broccoli (*Brassica oleracea*), *Eclipta prostrata*, or a combination comprising one or more of the foregoing compounds.

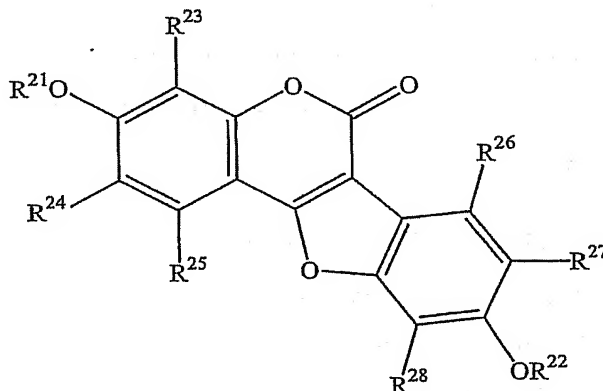
25 An ester of coumestrol is preferably a glycoside of coumestrol. There is no particular limit on the monosaccharide or polysaccharide used to form the glycoside of coumestrol. Suitable monosaccharides sugars include, for example, glucose, glucuronic acid, mannose, fructose, galactose, xylose, rutinose, rhamnose, and the like, and combinations comprising at least one of the foregoing monosaccharides.

Suitable polysaccharides include, for example, dimers, trimers, oligomers, and polymers formed from one or more of the above monosaccharides.

A coumestrol analog includes, for example, 4-ethyl-7-hydroxy-3-(p-methoxyphenyl)-2H-1-benzopyran-2-one (wedelolactone), and the like.

5 The coumestrol may comprise an extract of *Taraxacum mongolicum*, alfalfa sprout (*Medicago sativa*), broccoli (*Brassica oleracea*), *Eclipta prostrata*, or the like as a source of coumestrol, a pharmaceutically acceptable salt or ester of coumestrol, a selectively substituted analog of coumestrol, or a combination comprising one or more of the foregoing compounds.

10 A selectively substituted analog of coumestrol has the formula



wherein R^{21} and R^{22} are independently hydrogen or C_1 - C_6 alkyl (preferably hydrogen); and R^{23} - R^{28} are independently hydrogen, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, or C_2 - C_6 acyl (preferably hydrogen), with the proviso that at least three of R^{23} - R^{28} are hydrogen. In
 15 a preferred embodiment, R^{21} and R^{22} are hydrogen; and R^{23} - R^{28} are independently hydrogen, methyl, ethyl, methoxy, ethoxy, acetyl, or propionyl, with the proviso that at least four of R^{23} - R^{28} are hydrogen.

Methods for synthesizing or isolating coumestrol, its pharmaceutically acceptable salts or esters, its selectively substituted analogs, are known in the art. See,
 20 for example, P. M. Dewick et al., *J. Chem. Soc. D* (1969), 9: 466-7; T. Kappe et al., *Z. Naturforsch., Teil B* (1974), 29(3-4): 292-3; R. Laschober et al., *Synthesis* (1990), 5: 387-8; F. A. Macias et al., *Phytochemistry* (1998), 50(1): 35-46; K. Hiroya et al.,

Perkin 1 (2000), 24: 4339-4346; G. A. Kraus et al., *Journal of Organic Chemistry* (2000), 65(18): 5644-5646; and M. Okada et al., *Planta Med.* (2000), 66(6): 572-575.

When present, the coumestrol comprises greater than or equal to 0.5 weight percent, more preferably greater than or equal to about 1 weight percent, still more preferably greater than or equal to about 2 weight percent, even more preferably greater than or equal to about 5 weight percent, even more preferably greater than or equal to about 10 weight percent, still more preferably greater than or equal to about 20 weight percent of the total weight of the composition. Compositions containing as much as 50 weight percent, or even as much as 100 weight percent of coumestrol are contemplated.

Supplementary active ingredients may also be incorporated into the compositions and preparations. For example, administration of wogonin, isoliquiritigenin, coumestrol, and mixture comprising one or more of the foregoing phytoestrogens in combination with other anti-cancer agents is expected to stimulate anti-cancer activity.

In one embodiment, the composition may comprise: greater than or equal to 0.5 weight percent of a phytoestrogen selected from wogonin, isoliquiritigenin, coumestrol, and combinations comprising one or more of the foregoing phytoestrogens; and at least one anti-cancer agent. In such compositions, the phytoestrogen selected from wogonin, isoliquiritigenin, coumestrol, and combinations thereof, may be present in an amount of about 0.5 to about 50 weight percent of the total weight of active ingredients in the composition. Within this range, the amount may be greater than or equal to about 1, 2, 5, or 10 weight percent. Also within this range, the amount may be up to about 40, 30, or 20 weight percent. While the above weight percents are based on the total weight of active ingredients in the composition, they may, alternatively, be based on the total weight of phytoestrogen in the composition.

There is no particular limitation on the anti-cancer agent employed. Suitable anti-cancer agents include, for example, oridonin, indirubin, taxol, cis-platin, camptothecin, vincristine, monocrotaline, Maytansine, homoharringtonine,

colchicine, irisquinone A, irisquinone B, irisquinone C, acronycine, matrin, oxymatrin, curcumin, paricine, pariphyllin, and the like, and combinations comprising one or more of the foregoing anti-cancer agents. Preferred anti-cancer agents include oridonin.

5 Suitable anti-cancer agents also include, for example, an extract of a plant selected from *Rabdosia rubescens*, *Panax pseudo-ginseng* Wall, *Ganoderma lucidum* Karst, *Scutellaria baicalensis* Georgi, *Glycine max*, *Curcuma longa*, and the like, and combinations comprising one or more of the foregoing plants. An extract of *Rabdosia rubescens* may comprise oridonin; an extract of *Humulus lupulus* may comprise
10 lupulone; an extract of *Panax pseudo-ginseng* Wall may comprise a gensenoside; an extract of *Scutellaria baicalensis* Georgi may comprise baicalin; an extract of *Glycine max* may comprise a soy flavonoid, a soy isoflavonoid, or both; and an extract of *Curcuma longa* may comprise curcumin.

 The anti-cancer agent may comprise, for example, about 1 to about 10 parts by
15 weight of an extract of *Rabdosia rubescens*; about 10 to about 40 parts by weight of an extract of *Panax pseudo-ginseng* Wall; about 100 to about 500 parts by weight of an extract of *Ganoderma lucidum* Karst; about 10 to about 100 parts by weight of an extract of *Scutellaria baicalensis* Georgi; about 10 to about 100 parts by weight of an extract of *Glycine max*; and about 10 to about 100 parts by weight of an extract of
20 *Curcuma longa*.

 The anti-cancer agent may comprise, for example, an extract of *Humulus lupulus*; and an extract of a plant selected from the group consisting of *Panax pseudo-ginseng* Wall, *Ganoderma lucidum* Karst, *Scutellaria baicalensis* Georgi, *Glycine max*, *Curcuma longa*, and combinations comprising one or more of the foregoing
25 plants.

 The anti-cancer agent may comprise about 1 to about 10 parts by weight of an extract of *Humulus lupulus*; about 10 to about 40 parts by weight of an extract of *Panax pseudo-ginseng* Wall; about 100 to about 500 parts by weight of an extract of *Ganoderma lucidum* Karst; about 10 to about 100 parts by weight of an extract of
30 *Scutellaria baicalensis* Georgi; about 10 to about 100 parts by weight of an extract of

Glycine max; and about 10 to about 100 parts by weight of an extract of *Curcuma longa*.

The anti-cancer agent may be present at about 1 to about 90 weight percent of the total weight of active ingredients in the composition. Within this range, the anti-cancer agent amount may be greater than or equal to about 2, 5, or 10 weight percent. Also within this range, the anti-cancer agent amount may be up to about 80, 70, 50, or 25 weight percent.

In addition to an anti-cancer agent, the composition may, optionally, further comprise an immune stimulant. There is no particular limitation on the immune stimulant employed. Suitable immune stimulants include, for example, ginsenosides, ferulic acid, mannan, synanthrin, eleutheroside A, eleutheroside B, eleutheroside C, eleutheroside D, eleutheroside E, gynoside, beta-pachyman, inulin, glycoproteins, interferones, gamma-globulins, polysaccharides from *Ganoderma lucidum*, and the like, and combinations comprising one or more of the foregoing immune stimulants. Suitable immune stimulants further include, for example, extracts of *Ganoderma lucidum*, *Coriolus versicolor*, *Poria cocos*, and the like, and combinations comprising one or more of the foregoing extracts. Preferred immune stimulants include beta-pachyman.

The immune stimulant, when present, is employed at about 1 to about 90 weight percent of the total weight of active ingredients in the composition. Within this range, the immune stimulant amount may be greater than or equal to about 2, 5, 10, 20, or 50 weight percent. Also within this range, the immune stimulant amount may be up to about 80, 70, or 60 weight percent.

In a preferred embodiment, the composition comprises: greater than about 0.5 weight percent of a phytoestrogen selected from wogonin, isoliquiritigenin, coumestrol, or a combination comprising one or more of the foregoing compounds; an anti-cancer agent selected from oridonin, colchicine, vincristine, camptothecin, maytansine, taxol, and combinations comprising one or more of the foregoing anti-cancer agents; and an immune stimulant selected from ginsenosides, mannan, synanthrin, eleutheroside A, eleutheroside B, eleutheroside C, eleutheroside D,

eleutheroside E, gynosides, beta-pachyman, interferon, and combinations comprising one or more of the foregoing immune stimulants. In this embodiment, the composition preferably comprises: about 1 to about 40 weight percent of a compound selected from wogonin, isoliquiritigenin, coumestrol, and combinations comprising
5 one or more of the foregoing compounds; about 0.05 to about 5 weight percent of a compound selected from oridonin, camptothecin, vincristine, Indirubin, colchicine, ginsenosides, and combinations comprising one or more of the foregoing compounds; and about 10 to about 98 weight percent of a compound selected from beta-pachyman, mannan, synanthrin, gynosides, and combinations comprising one or more of the
10 foregoing compounds; wherein all weight percents are based on the total weight of the composition.

In another preferred embodiment, the composition comprises a phytoestrogen selected from the group consisting of wogonin, isoliquiritigenin, coumestrol, and combinations comprising one or more of the foregoing compounds; oridonin; and
15 beta-pachyman. In this embodiment, the composition preferably comprises: about 1 to about 30 weight percent of wogonin, isoliquiritigenin, coumestrol, or a combination comprising at least one of the foregoing compounds; about 0.1 to about 5 weight percent of oridonin; and about 20 to about 90 weight percent of beta-pachyman; wherein all weight percents are based on the total weight of the composition.

20 As the composition may be defined as comprising multiple components, it will be understood that each component is chemically distinct, particularly in the instance that a single chemical compound may satisfy the definition of more than one component.

Wogonin, isoliquiritigenin, coumestrol, their pharmaceutically acceptable salts
25 or esters, or their selectively substituted analogs may be isolated from natural sources or synthesized according to known methods, as described above. Purities of these compounds, as employed in the composition, may vary according to their method of isolation or synthesis, but purities of about 5 percent to greater than 99 percent may be suitable for use in the composition.

The phytoestrogens may be in the form of a pharmaceutically acceptable composition. Methods for the formulation of pharmaceutically acceptable compositions are generally known. The subject pharmaceutical formulations may comprise one or more non-biologically active compounds, i.e., excipients, such as stabilizers (to promote long term storage), emulsifiers, binding agents, thickening agents, salts, preservatives, and the like, depending on the route of administration.

For oral administration, the wogonin, isoliquiritigenin, coumestrol, their pharmaceutically acceptable salts or esters, their selectively substituted analogs, or the like, or combinations comprising one or more of the foregoing may be administered with an inert diluent or with an assimilable edible carrier, or incorporated directly with the food of the diet. The formulations may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspension syrups, wafers, and the like. The tablets, troches, pills, capsules and the like may also contain the following: a binder, such as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agents, such as sucrose, lactose or saccharin; a flavoring agent such as peppermint, oil of wintergreen, or the like flavoring. When the dosage unit is a capsule, it may contain, in addition to materials of the above type, a liquid carrier.

Various other materials may also be present as coatings or to otherwise modify the physical form of the dosage unit. A syrup or elixir may contain sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Such additional materials should be substantially non-toxic in the amounts employed. Furthermore, the active agents may be incorporated into sustained-release preparations and formulations. Formulations for parenteral administration may include sterile aqueous solutions or dispersions, and sterile powders for the extemporaneous preparation of sterile, injectable solutions or dispersions. The solutions or dispersions may also contain buffers, diluents, and other suitable additives, and may be designed to promote the cellular uptake of the active agents in the composition, e.g., liposomes. Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent

with one or more of the various other ingredients described above, followed by sterilization. Dispersions may generally be prepared by incorporating the various sterilized active ingredients into a sterile vehicle that contains the basic dispersion medium and the required other ingredients from those listed above. In the case of
5 sterile powders used to prepare sterile, injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solutions. Pharmaceutical formulations for topical administration may be especially useful for localized treatment. Formulations for topical treatment included
10 ointments, sprays, gels, suspensions, lotions, creams, and the like. Formulations for topical administration may include known carrier materials such as isopropanol, glycerol, paraffin, stearyl alcohol, polyethylene glycol, and the like. The pharmaceutically acceptable carrier may also include a known chemical absorption promoter. Absorption promoters include, for example, dimethylacetamide (U.S. Pat.
15 No. 3,472,931 to Stoughton), trichloroethanol or trifluoroethanol (U.S. Pat. No. 3,891,757 to Higuchi), certain alcohols and mixtures thereof (British Patent Nos. 1,001,949 to Meyer and 1,464,975 to Astra Lakemedel). Except insofar as any conventional media or agent is incompatible with the therapeutic active ingredients, its use in the therapeutic compositions and preparations is contemplated.

20 The composition may, optionally, be in an ingestible form, preferably a powder, a capsule, or a tablet. Alternatively, the composition may be in the form of a suppository.

The pharmaceutical compositions described preferably contain about 0.5% to 100% by weight of active agent. Within this range, the compositions and preparation
25 may preferably comprise the active agent in an amount of at least about 1, 2, 5, 10, or 20 weight percent. Also within this range, the composition may preferably comprise the active agent in an amount of up to about 90, 80, 70, 60, or 50 weight percent. The amount of active compounds in such pharmaceutically useful compositions and preparations is such that a suitable dosage will be obtained.

Another embodiment is a method for the treatment of a human having cancer, an estrogen-related cancer, or other estrogen-related disorder. The method comprises treating a human in need of such treatment with a composition comprising a phytoestrogen selected from wogonin, isoliquiritigenin, coumestrol, or a combination
5 comprising one or more of the foregoing phytoestrogens. The terms "treating" and "treatment" as used herein refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, prevention of the occurrence of symptoms and/or their underlying cause, and improvement or remediation of damage. Thus, for example, the present method of "treating" an
10 estrogen-dependent disorder or cancer, as the term is used herein, encompasses both prevention of the disorder and treatment of the disorder in a clinically symptomatic individual.

By the terms "effective amount" or "pharmaceutically effective amount" or "an effective anti-estrogenic amount" of an agent as provided herein are meant a nontoxic
15 but sufficient amount of the agent to provide the desired prophylactic or therapeutic effect. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the age and general condition of the subject, the severity of the condition being treated, and the particular phytoestrogen employed and mode of administration, and the like. Thus, it is not possible to specify an exact "effective
20 amount". However, an appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

By "pharmaceutically acceptable carrier" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected phytoestrogen without causing any undesirable
25 biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

Cancer is the growth of new cells in the body wherein the new cells typically have adverse effects in the body. Cancer is characterized by an increase in the number of abnormal, or neoplastic, cells derived from a normal tissue which proliferate to
30 form a tumor mass, the invasion of adjacent tissues by these neoplastic tumor cells, and the generation of malignant cells which eventually spread via the blood or

lymphatic system to regional lymph nodes and to distant sites via a process called metastasis. In a cancerous state, a cell proliferates under conditions in which normal cells would not grow. Cancer manifests itself in a wide variety of forms, characterized by different degrees of invasiveness and aggressiveness.

5 Administration of a phytoestrogen such as wogonin, isoliquiritigenin, coumestrol, or combinations thereof, is effective to provide anti-cancer activity. It is believed that the general anti-cancer activity of wogonin, isoliquiritigenin, coumestrol is related to their activity as COX-2 inhibitors. COX-2 is a key inducible enzyme in the conversion of arachidonic acid to prostaglandins and other eicosanoids. COX-2
10 expression can be induced by a variety of factors, including, for example, growth factors, interleukin-1, and tumor promoting factors. The enzyme is expressed in a number of tumor cells, and human cancers, among which is prostate cancer. COX-2 inhibitors are known have use as anti-cancer therapeutics.

In addition to general anti-cancer activity, the phytoestrogens wogonin,
15 isoliquiritigenin, and coumestrol are useful as anti-hormone-related cancer agents. Hormone-related cancers include, for example, bladder cancer, bone cancer, breast cancer, colon cancer, endometrial cancer, lung cancer, ovarian cancer, prostate cancer, testicular cancer, and thyroid cancer.

In the treatment of cancer, the phytoestrogen compositions may be
20 administered orally, parenterally, transdermally, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term "parenteral" as used herein is intended to include subcutaneous, intravenous, and intramuscular injection. The amount of active compound administered will, of course,
25 be dependent on the subject being treated, the subject's weight, the manner of administration and the judgment of the prescribing physician. Generally, however, the dosage of phytoestrogen will be about 0.01 mg/kg/day to about 1000 mg/kg/day, preferably about 0.01 mg/kg/day to about 300 mg/kg/day, more preferably about 1 mg/kg/day to about 300 mg/kg/day. When the phytoestrogen is used in combination
30 with an anti-cancer agent, the dosage of the phytoestrogen will be about 0.01

mg/kg/day to about 1000 mg/kg/day, preferably about 0.01 mg/kg/day to about 300 mg/kg/day, more preferably about 1 mg/kg/day to about 300 mg/kg/day, and the dosage of anti-cancer agent will be about 0.1 ug/kg/day to about 100 mg/kg/day, preferably about 0.3 ug/kg/day to about 50 mg/kg/day, more preferably about 0.01
5 mg/kg/day to about 50 mg/kg/day. When the phytoestrogen composition comprises an immune stimulant, the dosage of immune stimulant will be about 1 mg/kg/day to about 5000 mg/kg/day, more preferably about 5 mg/kg/day to about 1000 mg/kg/day

The phytoestrogens wogonin, isoliquiritigenin, and coumestrol may also be used in the treatment of other estrogen-related disorders including, for example, bone
10 loss, bone fractures, osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, rheumatoid arthritis, osteoarthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, cartilage degeneration, endometriosis, uterine fibroid disease, hot flashes, cardiovascular disease, impairment
15 of cognitive function, cerebral degenerative disorders, restenosis, gynecomastia, vascular smooth muscle cell proliferation, obesity, incontinence, and combinations thereof. As used herein, estrogen-related disorder also includes the symptoms of menopause, the transition from the reproductive stage to the non-reproductive stage of a woman's life, characterized primarily by the cessation of menstruation. Symptoms
20 of menopause include, for example, hot flashes, sweating secondary to vasomotor instability, psychological and emotional symptoms of fatigue, insomnia, irritability and nervousness, lack of sleep, dizziness, cardiac symptoms; the incidence of heart disease increases, nausea, constipation, diarrhea, arthralgia, myalgia, and combinations of the foregoing symptoms.

25 Of particular interest is the treatment and prevention of osteoporosis. Osteoporosis, or loss of bone density, results in increased bone fractures and vertebral column collapse. Bone loss often begins around age 35. This loss accelerates during menopause, which generally occurs around age 45 to 55. Bone mass losses average about 1-2% each year after menopause. The primary sites are the vertebrae, which
30 show anterior collapse resulting in stooping and backache, the hips and the wrist.

Osteoporosis develops over decades and is related to peak bone mass, as well as to the degree of bone loss.

In the treatment of an estrogen-related disorder, the phytoestrogen compositions may be administered orally, parenterally, transdermally, rectally, 5 nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term "parenteral" as used herein is intended to include subcutaneous, intravenous, and intramuscular injection. The amount of active compound administered will, of course, be dependent on the subject being treated, the subject's 10 weight, the manner of administration and the judgment of the prescribing physician. Generally, however, the dosage of phytoestrogen will be about 0.1 mg/kg/day to about 1000 mg/kg/day, more preferably about 0.1 mg/kg/day to about 300 mg/kg/day.

The invention is further illustrated by the following non-limiting examples.

GENERAL EXPERIMENTAL

Wogonin was extracted from 7 grams of a multi-component botanical extract 15 composition containing extracts of *Panax pseudo-ginseng* Wall, *Isatis Indigotica* Fort, *Ganoderma lucidium* Karst, *Dendrathera morifolium* Tzvel, *Glycyrrhiza glabra* L, *Sculetaria bailcalensis* Georgi, *Rabdosia rubescens*, and *Serenoa repens*. The powder was dissolved in 150 milliliters of acetone using a soxhlet extractor for one hour. The liquid phase extract was further purified by silica gel column chromatography using a 20 solvent system of 2:1 cyclohexane:acetone. About 25 milligrams of the yellow powder was obtained from fractions 11-13 (8 milliliters per fraction). The powder was further re-crystallized from absolute ethanol to yield yellow crystals.

Figure 1 is a high performance liquid chromatogram showing the location of wogonin in the elution profile. The chromatogram was obtained with a Shimadzu SPD-M10A chromatograph using a C18 reverse phase column and two solvent systems of water and acetonitrile in 0.1% trifluoroacetic acid.

The chemical structure and molecular weight of the yellow crystal was determined by total (Fig. 2(b)) and DEPT (Fig. 2(a)) ^{13}C NMR (d-4 methanol solvent

analyzed on Varian^{UNITY} Inova 400 system). The sample was also analyzed by electron ionization with a Hewlett Packard VG 7070; the mass spectrum is shown in Figure 3 and indicates a molecular weight of 284, consistent with wogonin.

Isoliquiritigenin was purified from the flavonoid fraction of *Glycyrrhiza*
5 *glabra* concentrated powder (purchased from Shanghai Zhao Wei Technology Development Co.) extracted by absolute ethanol. About 1 gram of the flavonoid concentrate (dissolved in 5 milliliters of water) was passed through Sephadex LH-20 column (2.5x30 millimeters) and eluted by a gradient of methanol-water mixed solvent. Crude isoliquiritigenin was obtained at fraction 27 (10 milliliters per
10 fraction). The crude product was further purified by silica gel chromatography eluted by mixed solvent of methylene chloride: methanol (5:1).

The chemical structure and the molecular weight of isoliquiritigenin were determined by the absorption spectrum (Fig. 5) associated with an HPLC separation (Fig. 4) performed on a Shimadzu SPD-M10A chromatograph, as well as the ¹³C
15 NMR spectra (Fig. 6(a), DEPT; Fig. 6(b), total) and mass spectrum (Fig. 7). The absorption spectrum was identical to that of the reference compound isoliquiritigenin purchased from Sigma Chemical Co, (St. Louis, Missouri).

The anti-cancer activities of wogonin and isoliquiritigenin were evaluated by determining their abilities to inhibit cancer cell growth, to modulate the cancer cell
20 cycle, and to activate estrogen receptors.

Cancer cell lines: LNCaP, DU-145, and MCF-7 cells were purchased from the American Type Culture Collection. PTX 10, a taxol-resistant ovarian cancer cell line, was obtained from the Brander Cancer Research Laboratory, New York Medical College. Cells were maintained in RPMI 1640 culture media supplemented with 10%
25 heat-inactivated FBS, 5 millimolar glutamine, 50 units/milliliter of penicillin G, and 50 grams/milliliter of streptomycin. The cells were routinely seeded at 1X 10⁵ cells/milliliter in T-75 flasks, allowed to attach overnight, then treated with the herbal extract. At different times, cells were harvested by trypsinization.

EXAMPLE 1

This example demonstrates the activity of wogonin and isoliquiritigenin in inhibiting the growth of the hormone-sensitive prostate cancer cell line, LNCaP.

The MTT assay (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was used to count viable cells. The assay reagents were purchased from Boehringer Mannheim (Roche Diagnosis Corp, Indianapolis, Indiana). In this assay, the tetrazolium dye MTT is cleaved to form formazan by metabolically active cells and exhibits a strong red absorption band at 550-618 nm. The protocol for the cell viability assay was provided by the manufacturer and modified in our laboratory as described below.

Prostate or breast cancer cells were seeded in 96 well microtiter plates at a concentration of 3×10^3 cells per well (MCF-7; breast cancer cells) or 6×10^3 cells per well (LNCaP or DU145; prostate cancer cells), in a volume of 100 microliters of cell culture medium. After 24 hours, 20 microliter aliquots of the compounds at various concentrations were added to the attached cells. Each concentration was repeated in 3 different wells to obtain mean values. To eliminate any solvent effect, 20 microliters of the solvent used in the preparation of the highest concentration of the compounds (a maximum of 0.5 % by volume of dimethylsulfoxide (DMSO)) was added to the control cells in each well. The plates were incubated at 37°C in a CO₂ incubator for 72 hours. At the end of day 3 (after 72 hours), the culture medium was carefully removed without disturbing the cells and replaced by 100 microliters of fresh cell medium. Ten microliters of MTT reagent was added to each well and the plates were incubated again in a CO₂ incubator at 37°C for 4 hours. One hundred microliters of sodium dodecylsulfate (SDS) solubilizing reagent (from Boehringer Mannheim) was added to each well. The plate was allowed to stand overnight in the CO₂ incubator and read by an ELISA Reader (EL800, Bio-Tek Instruments, Inc.) at a wavelength of 570 nm. The percent cell viability was calculated according to the equation below:

$$V = 100 \left(\frac{A_{\text{control}} - A_{\text{treated}}}{A_{\text{control}}} \right)$$

where V is the percent cell viability, A_{control} is the absorption of the control cells, and A_{treated} is the absorption of the treated cells.

Figures 8 and 9 demonstrate the activity of wogonin and isoliquiritigenin respectively, in inhibiting the growth of LNCaP (androgen-dependent) and DU-145 (androgen-independent) prostate cancer cells. It is apparent that the inhibition of cell growth is dose dependent. The concentration of the compounds resulting in 50% inhibition of cancer cell growth, defined as ED₅₀, was determined by linear interpolation. ED₅₀ values for the two compounds obtained from these measurements are shown in Columns 1 and 2 of Table 1.

Table 1. ED₅₀ values from MTT Assay as a Function of Compound and Cell Type

	LNCaP ($\mu\text{g/ml}$)	DU145 ($\mu\text{g/ml}$)	MCF7 ($\mu\text{g/ml}$)
Wogonin	10.00	18.6	NA
isoliquiritigenin	3.51	7.60	3.25

10 EXAMPLE 2

This example demonstrates the activity of isoliquiritigenin in inhibiting the growth of the breast cancer cell line, MCF-7.

The same protocols described in Example 1 were used to evaluate the effects of isoliquiritigenin on MCF-7 cells. MCF-7 is a breast cancer cell line that expresses estrogen receptors. Therefore it is a good model to study the effect of the anti-cancer agents on estrogen-receptor positive breast cancer.

Figure 9 shows the MTT assay curves for isoliquiritigenin with MCF-7. The data show that isoliquiritigenin inhibited the growth of MCF-7 cells, and dosage-dependent curves were observed. ED₅₀ values are given in Column 3 of Table 1, above.

EXAMPLE 3

This example demonstrates modulation of the LNCaP cell cycle by wogonin and isoliquiritigenin. LNCaP cells have a hormone-dependent cell cycle.

Sample preparation for cell cycle measurement: Cultured cells ($2-4 \times 10^6$ cells) were exposed to two concentrations each of wogonin and isoliquiritigenin for 24-48 hours in 12.5 cm^2 area flasks before being harvested. The cells were washed with phosphate buffered saline (PBS) and fixed in ice-cold 70% ethanol. Aliquots of fixed cells were rehydrated in PBS and stained with 1.0 microgram/milliliter DAPI (4,6-diamidino-2-phenylindole from Eastman Kodak, Rochester, NY), and dissolved in 10 millimolar piperazine-N,N-bis-2-ethane-sulfonic acid buffer (Calbiochem, La Jolla, CA) containing 100 millimolar NaCl, 2mM MgCl_2 and 0.1% Triton X-100 (Sigma) at pH 6.8 as previously described by Halicka et al. (H. D. Halicka, B. Ardelt, G. Juan, A. Mittelman, S. Chen, F. Traganos and Z. Darzynkiewicz, "Apoptosis and Cell Cycle Effects Induced by Extracts of the Chinese Herbal Preparation of PC SPES", *International J. of Oncology* (1997), 11: 437-448).

The cellular DNA content was measured with an ELITE ESP flow cytometer (Coulter Inc., FL.) using UV laser illumination. The multicycle program was used to deconvolute the DNA frequency histograms to estimate the frequency of cells in different phases of the cell cycle.

Figure 10 displays the DNA histograms of LNCaP cells in the presence and absence of wogonin at 3 micrograms/milliliter after 24 hours. It is evident that there was a change at the G1 phase as shown by the arrow bar. Data analysis revealed the increase in G1 phase was proportional to wogonin concentration.

Similar measurements were conducted for isoliquiritigenin. Figure 11 summarizes the effects of wogonin and isoliquiritigenin on G1, S, and G₂M phases of the LNCaP cell cycle. The data show that wogonin induced a G1 phase arrest, and isoliquiritigenin induced a G₂M phase arrest. A prolongation in either G1 or G₂M phases leads to the suppression of LNCaP cell proliferation.

EXAMPLE 4

This example demonstrates modulation of the DU-145 cell cycle by wogonin and isoliquiritigenin.

The protocol described in Example 3 was used to study the effect of wogonin on the hormone-independent prostate cancer cell line DU-145. The results are presented in Figure 12 and show that wogonin prolonged the G₂M phase of DU-145. A prolongation in the G₂M phase leads to the suppression of DU-145 cell proliferation.

EXAMPLE 5

The estrogenic activity of wogonin and isoliquiritigenin were demonstrated by determining their ability to activate estrogen receptors (subclass alpha and beta).

HEK 293 cells (ATCC CRL-1573) were transfected with an expression vector for hER α and hER β respectively, and an ERE-LUC reporter gene (plus a TK-LUC reporter for normalization) following the protocol of Yoon et al ("Differential activation of wild-type and variant forms of estrogen receptor α by synthetic and natural estrogenic compounds using a promoter containing three estrogen-responsive elements", *J. Steroid Biochem. & Molecular Biology* (2000), 28: pages 25-32).

Cells were then separately exposed to wogonin or isoliquiritigenin at concentrations of 0, 0.07, 0.02, 0.08, 0.3, 0.7, 1.4 (or 4) and 9 μ g/ml for 20 hours and then cell lysates were assayed for reporter gene expression (Tzukerman et al. "Human estrogen receptor transactivational capacity is determined by both cellular and promoter content and mediated by two functionally distinct intermolecular regions", *Mol. Endocrinol.* (1994), 8: 21-30).

Figure 13 shows the dose-responsive behavior of ER α -Luc reporter gene activated by wogonin and isoliquiritigenin.

Figure 14 shows the dose-responsive behavior of ER β -Luc reporter gene activated by wogonin and isoliquiritigenin. It is very significant that wogonin and

isoliquiritigenin showed at least 10 times more capability in activating ER β -Luc reporter gene than the ER α -Luc reporter gene.

EXAMPLE 6

This example demonstrates the inhibition of COX-2 activity by
5 isoliquiritigenin.

COX is a bifunctional enzyme that exhibits both cyclooxygenase and peroxidase activities. The cyclooxygenase activity is responsible for the oxidation of arachidonic acid to Prostaglandin G₂ (PGG₂) and the peroxidase activity is responsible for the subsequent reduction of PGG₂ to the corresponding alcohol, PGH₂. Some
10 methods used to determine COX inhibitor activity include measuring uptake of oxygen using an oxygraph, measuring the conversion of radioactive arachidonic acid, or measuring the prostaglandins formed from PGH₂ (using immunoassay techniques).

These experiments employed the ovine COX-2 Inhibitor Screening Assay commercially available as catalog no. 760101 from Cayman Chemical (Ann Arbor,
15 MI 48108). This immunoassay uses an antibody to that binds all major prostaglandins to measure quantitatively the amount of PGF_{2 α} produced in the COX reaction using arachidonic acid as a substrate. The final volume of the reactions as described below is typically 1.15 ml. In brief, COX-1 or COX-2 (e.g., 20 μ l of a solution of from 1 to 100 units/ml COX, where a unit is defined as the amount of enzyme that consumes
20 one nanomole of oxygen per minute at 37°C in 0.1 M Tris-HCl buffer, pH 8.0 containing 100 μ M arachidonate, 5 mM EDTA, 2 mM phenol and 1 μ M hematin) is first mixed with a buffer (e.g., 0.1 M Tris, pH 8.0, 5 mM EDTA, 2 mM phenol) and heme (e.g., 10 μ l of 10 mM heme) in a microfuge tube. The heme is a cofactor for COX that provides maximal activity in the assay. COX samples that contain an
25 inhibitor can be pre-mixed with the inhibitor (e.g., 1-100 μ M) prior to adding substrate. The arachidonic acid substrate (e.g., 10 μ l of a 10 mM solution) can be then added to the COX/heme/inhibitor mixture for a time and at a temperature sufficient for the reaction to proceed to produce a detectable product (e.g., 2 minutes at 37°C). The reaction can be quenched with acid (e.g., 50 μ l of 1 M HCl).

Additionally stannous chloride (e.g., 100 μ l of a saturated solution) can be added to convert the PGH_2 produced to the more stable $\text{PGF}_{2\alpha}$ for the purpose of quantification of prostaglandin. The prostaglandin produced in the reactions is typically quantified using an enzyme immunoassay.

5 The enzyme immunoassay to detect prostaglandin can conveniently be performed in a 96 well plate using an antibody to detect prostaglandin. As controls, prostaglandin standards and COX 100% activity samples (no inhibitor) can be measured. Samples used for background correction can also be used. Controls and reactions performed with COX and the inhibitors of interest are incubated with
10 prostaglandin screening antiserum in an amount sufficient to detect the prostaglandin produced in the COX reactions (e.g., 50 μ l of antiserum diluted in 6 ml of a suitable EIA buffer). The reactions can be incubated for a time and temperature sufficient to allow interaction of the antiserum and the prostaglandins (e.g., 18 hours at room temperature). When ready to develop the plate, the reactions are first washed and then
15 incubated with 200 μ l of Ellman's reagent for 60 minutes or so. The plate can be read at 405 to 420 nm on a plate reader. The prostaglandin standards are used to calculate a standard curve of prostaglandin concentration. The amount of prostaglandin in each sample with inhibitor is subtracted from the amount of prostaglandin in the 100% activity sample, divided by the amount of prostaglandin in the 100% activity sample,
20 and multiplied by 100 to give the percent inhibition. Graphing the percent inhibition vs. the inhibitor calculation allows the calculation of the IC_{50} value (the concentration at which there is 50% inhibition).

Methods of measuring the activity of a COX inhibitor are described in, for example, W. Xie, J. G. Chipman, D. L. Robertson, et al., "Expression of a mitogen-
25 responsive gene encoding prostaglandin synthase is regulated by mRNA splicing", *Proc. Natl. Acad. Sci. USA* (1991), 88: 2692-2696; K.M. Maxey, K.R. Maddipati, and J. Birkmeier, "Interference in enzyme immunoassays", *J. Clin. Immunoassay* (1992), 15: 116-120; P. Pradelles, J. Grassi, and J. A. Macclouf, "Enzyme immunoassays of eicosanoids using acetylcholinesterase as label: An alternative to radioimmunoassay",
30 *Anal. Chem.* (1985), 57: 1170-1173; Macclouf, J., Grassi, J., and Pradelles, P. Development of enzyme-immunoassay techniques for the measurement of

eicosanoids, *Prostaglandin and Lipid Metabolism in Radiation Injury* (1987), pages 355-364.

Figure 15 displays the dose-dependent inhibitory activity of isoliquiritigenin on COX-2 measured according to the procedure above. An IC_{50} of $10.5 \mu M$ was calculated from the data.

EXAMPLE 7: demonstrates the cytotoxicity of wogonin and isoliquiritigenin on an ovarian cancer cell line:

PTX 10, a taxol resistant ovarian cancer cell line, cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 mg/ml streptomycin, and 2mM L-glutamine (all from Gibco/BRL Life Technologies, Inc., Grand Island, NY) at $37.5^{\circ}C$ in an atmosphere of 5% CO_2 in air. At the onset of experiments the cultures were at the densities below 5×10^5 cells/ml and the cells were growing exponentially and asynchronously.

The MTT assay was performed to study the effect of wogonin and isoliquiritigenin on the cell growth of PTX 10. The MTT assay protocol is the same as that described in Example 1. Figures 16 and 17 display the inhibition curves of PTX cell line in the presence of wogonin and isoliquiritigenin respectively. A concentration- dependent inhibition was clearly observed in these two figures. The ED_{50} calculated from the inhibition curves are 1.56 $\mu g/mL$ and 3.32 $\mu g/mL$ for wogonin and isoliquiritigenin respectively as shown in Table 2.

Table 2 ED_{50}

	PTX 10 (taxol resistant Ovarian cancer cell)
Wogonin (I-16-2)	< 1.56 $\mu g/mL$
Isoliquiritigenin	3.32 $\mu g/mL$

Thus, wogonin and isoliquiritigenin may have utility for treating cancers that are resistant to treatment by other agents such as, for example, taxol.

EXAMPLE 8

A composition according to this disclosure was administered in capsules 6 times a day to two elderly volunteer patients diagnosed with prostate cancer. As a measure of the progress of the cancer, the bloodstream level of prostate-specific antigen (PSA), a substance produced by the prostate gland, was measured by standard methods. The results are shown in Table 3.

Table 3

Patient Age	Treatment prior to treatment with Composition 1	PSA at start of treatment, ng/mL	PSA after 1 month of treatment, ng/mL	PSA after 2 months of treatment, ng/mL	Percent PSA reduction after 1 month of treatment	Percent PSA reduction after 2 months of treatment
73	Castration plus Flutamide	80	40	6	50%	92.5%
79	Prostatectomy; no medication	120	64	18	46.7%	85%

As can be seen from table 1, a dramatic reduction in PSA levels is observed after 1 and 2 months of treatment with composition 1.

Compositions comprising wogonin, isoliquiritigenin, coumestrol and combinations thereof have both phytoestrogenic and anti-cancer activities. When used in cancer therapy, the compositions may comprise additional anti-cancer agents and/or immune stimulants. The anti-cancer activity is demonstrated by the ability of wogonin and isoliquiritigenin to inhibit the growth of cancer cell lines. The identification of isoliquiritigenin as a COX-2 inhibitor suggests that it has general anti-cancer activity. The identification of the phytoestrogenic activity of wogonin suggests that, in addition to general anti-cancer activity due to COX-2 inhibition, wogonin may be particularly useful in the treatment of hormone-related cancers. In

addition, wogonin may be used in the treatment of hormone-related disorders such as, for example, osteoporosis.

While the invention has been described with reference to a preferred embodiment, it will be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the scope of the invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from essential scope thereof. Therefore, it is intended that the invention not be limited to the particular embodiment disclosed as the best mode contemplated for carrying out this invention, but that the invention will include all embodiments falling within the scope of the appended claims.

All cited patents, patent applications, and other references are incorporated herein by reference in their entirety.

1. A method of treating a human in need of cancer treatment, comprising administering a composition comprising greater than 0.5 weight percent based on the total weight of the composition of wogonin, isoliquiritigenin, coumestrol, their pharmaceutically acceptable salts and esters, their selectively substituted analogs, or a combination of one or more of the foregoing compounds.

2. The method of Claim 1, wherein the cancer is prostate cancer, breast cancer, endometrial cancer, colon cancer, lung cancer, bladder cancer, testicular cancer, ovarian cancer, thyroid cancer, or bone cancer

3. The method of Claim 1, wherein the composition comprises a compound selected from the group consisting of wogonin, a pharmaceutically acceptable salt or ester of wogonin, a selectively substituted analog of wogonin, or a combination of one or more of the foregoing compounds.

4. The method of Claim 3, wherein the composition comprises a glycoside of wogonin; a selectively substituted analog of wogonin selected from the group consisting of genistein, biochanin, prunetin, scutellarein, daidzin, luteolin, apigenin, acacetin, 3,6,4-trihydroxyflavone, 7,3-dihydroxy-4,1-dimethoxy-isoflavone, 3R-2',3'-dihydroxy-7,4-dimethoxy-isoflavone; or a combination of one or more of the foregoing compounds.

5. The method of Claim 3, wherein the composition comprises an extract of an herb in the family *Scutellaria*.

6. The method of Claim 3, wherein treating comprises administering a dosage of about 0.001 mg/kg/day to about 300 mg/kg/day of wogonin.

7. The method of Claim 3, wherein the composition further comprises isoliquiritigenin, coumestrol, or a combination of one or more of the foregoing compounds.

8. The method of Claim 1, wherein the composition comprises a compound selected from the group consisting of isoliquiritigenin, a pharmaceutically acceptable salt or ester of isoliquiritigenin, a selectively substituted analog of isoliquiritigenin, or a combination of one or more of the foregoing compounds.

9. The method of Claim 8, wherein the composition comprises phloretin, 4,2,4'-trihydroxychalcone, or a combination of one or more of the foregoing compounds.

10. The method of Claim 8, wherein the composition comprises an extract of *Glycyrrhiza uralensis*, *Glycyrrhiza glabra* or a combination of one or more of the foregoing compounds.

11. The method of Claim 8, wherein the composition further comprises wogonin, coumestrol, or a combination of one or more of the foregoing compounds.

12. The method of Claim 8, wherein treating comprises administering a dosage of about 0.001 mg/kg/day to about 300 mg/kg/day of isoliquiritigenin.

13. The method of Claim 1, wherein the composition comprises a compound selected from the group consisting of coumestrol, a pharmaceutically acceptable salt or ester of coumestrol, a selectively substituted analog of coumestrol, a, or a combination of one or more of the foregoing compounds.

14. The method of Claim 13, wherein the composition comprises an extract of a plant selected from the group consisting of *Taraxacum mongolicum*, *Medicago sativa*, *Brassica oleracea*, or *Eclipta prostrata* and combinations of one or more of the foregoing plant extracts.

15. The method of Claim 13, wherein the composition further comprises wogonin, isoliquiritigenin, or a combination of one or more of the foregoing compounds.

16. The method of Claim 13, wherein treating comprises administering a dosage of about 0.001 mg/kg/day to about 300 mg/kg/day of coumestrol.

17. The method of Claim 1, wherein the composition further comprises an anti-cancer agent.

18. The method of Claim 17, wherein the anti-cancer agent is selected from the group consisting of oridonin, indirubin, taxol, cis-platin, camptothecin, vincristine, monocrotaline, Maytansine, homoharringtonine, colchicine, irisquinone A, irisquinone B, irisquinone C, acronycine, matrin, oxymatrin, curcumin, paricine, 5 pariphyllin, and combinations comprising one or more of the foregoing anti-cancer agents.

19. The method of Claim 17, wherein the composition further comprises an immune stimulant.

20. The composition of Claim 19, wherein the immune stimulant is selected from the group consisting of ginsenosides, ferulic acid, mannan, synanthrin, eleutheroside A, eleutheroside B, eleutheroside C, eleutheroside D, eleutheroside E, gynoside, beta-pachyman, inulin, glycoproteins, interferones, γ -globulins, extracts of 5 *Ganoderma lucidum*, extracts of *Coriolus versicolor*, extracts of *Poria cocos*, and combinations comprising one or more of the foregoing immune stimulants.

21. A composition, comprising:

greater than or equal to about 0.5 weight percent based on the total weight of the composition of a compound selected from wogonin, isoliquiritigenin, coumestrol, their pharmaceutically acceptable salts or esters, their selectively substituted analogs, 5 and combinations comprising one or more of the foregoing compounds; and

at least one anti-cancer agent.

22. The composition of Claim 21, wherein the anti-cancer agent is selected from the group consisting of oridonin, indirubin, taxol, cis-platin, camptothecin, vincristine, monocrotaline, Maytansine, homoharringtonine, colchicine, irisquinone A, irisquinone B, irisquinone C, acronycine, matrin, oxymatrin, curcumin, paricine, 5 pariphyllin, and combinations comprising one or more of the foregoing anti-cancer agents.

23. The composition of Claim 21, wherein the anti-cancer agent comprises: an extract of *Rabdosia rubescens*; and an extract of a plant selected from the group consisting of *Panax pseudo-ginseng* Wall, *Ganoderma lucidum* Karst, *Scutellaria baicalensis* Georgi, *Glycine max*, *Curcuma longa*, and combinations comprising at 5 least one of the foregoing plants.

24. The composition of Claim 23, wherein the anti-cancer agent comprises:
about 1 to about 10 parts by weight of an extract of *Rabdosia rubescens*;
about 10 to about 40 parts by weight of an extract of *Panax pseudo-ginseng* Wall;
5 about 100 to about 500 parts by weight of an extract of *Ganoderma lucidum* Karst;
about 10 to about 100 parts by weight of an extract of *Scutellaria baicalensis* Georgi;
about 10 to about 100 parts by weight of an extract of *Glycine max*; and
10 about 10 to about 100 parts by weight of an extract of *Curcuma longa*.

25. The composition of Claim 21, wherein the anti-cancer agent comprises:
an extract of *Humulus lupulus*; and an extract of a plant
selected from the group consisting of *Panax pseudo-ginseng*
Wall, *Ganoderma lucidum* Karst, *Scutellaria baicalensis*
Georgi, *Glycine max*, *Curcuma longa*, and combinations
comprising one or more of the foregoing plants.
26. The composition of Claim 25, wherein the anti-cancer agent comprises:
about 1 to about 10 parts by weight of an extract of *Humulus lupulus*;
about 10 to about 40 parts by weight of an extract of *Panax pseudo-ginseng*
Wall;
about 100 to about 500 parts by weight of an extract of *Ganoderma lucidum*
Karst;
about 10 to about 100 parts by weight of an extract of *Scutellaria baicalensis*
Georgi;
about 10 to about 100 parts by weight of an extract of *Glycine max*; and
about 10 to about 100 parts by weight of an extract of *Curcuma longa*.
27. The composition of Claim 21, further comprising an immune
stimulant.
28. The composition of Claim 27, wherein the immune stimulant is
selected from the group consisting of ginsenosides, ferulic acid, mannan, synanthrin,
eleutheroside A, eleutheroside B, eleutheroside C, eleutheroside D, eleutheroside E,
gynoside, beta-pachyman, inulin, glycoproteins, interferones, γ -globulins, extracts of
Ganoderma lucidum, extracts of *Coriolus versicolor*, extracts of *Poria cocos*, and
combinations comprising one or more of the foregoing immune stimulants.

29. A composition, comprising:

a compound selected from the group consisting of wogonin, isoliquiritigenin, coumestrol, and combinations comprising one or more of the foregoing compounds;

oridonin; and

5 beta-pachyman.

30. A composition, comprising:

about 1 to about 30 weight percent of wogonin, isoliquiritigenin, coumestrol, or a combination comprising one or more of the foregoing compounds;

about 0.1 to about 5 weight percent of oridonin; and

5 about 20 to about 90 weight percent of beta-pachyman;

wherein all weight percents are based on the total weight of the composition.

31. A method of treating a human in need of treatment for an estrogen-related disorder, comprising administering a composition comprising greater than 0.5 weight percent based on the total weight of the composition of wogonin, its pharmaceutically acceptable salts and esters, its selectively substituted analogs, or a
5 combination of one or more of the foregoing compounds.

32. The method of Claim 31, wherein the estrogen-related disorder is selected from the group consisting of bone loss, bone fractures, osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, rheumatoid arthritis, osteoarthritis,
5 periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, cartilage degeneration, endometriosis, uterine fibroid disease, hot flashes, cardiovascular disease, impairment of cognitive function, cerebral degenerative disorders, restenosis, gynecomastia, vascular smooth muscle cell proliferation, obesity, incontinence, the symptoms of menopause, and combinations
10 comprising one or more of the foregoing disorders.

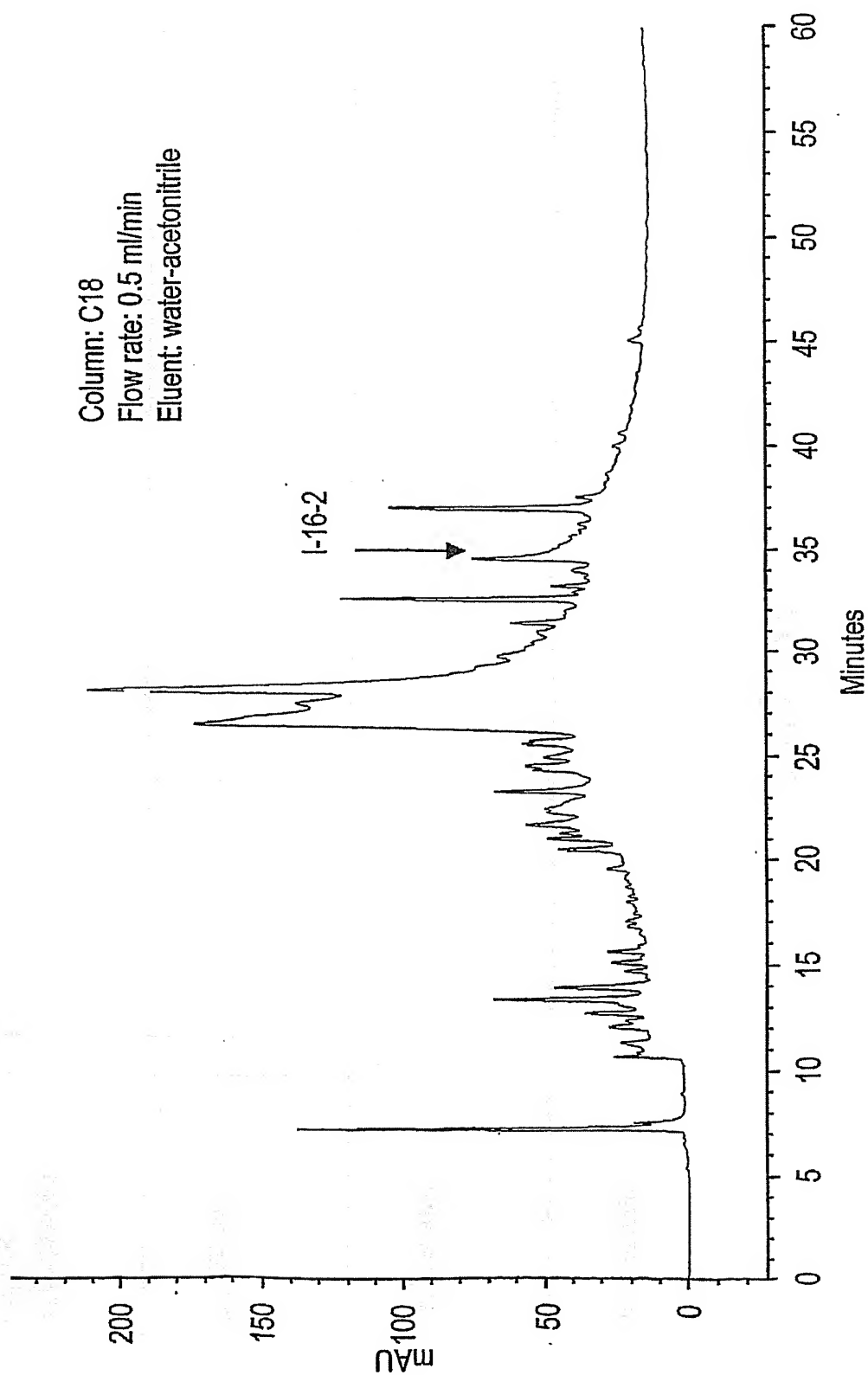
33. The method of Claim 31, wherein the wogonin comprises a glycoside of wogonin; a selectively substituted analog of wogonin selected from the group consisting of genistein, biochanin, prunetin, scutellarein, daidzin, luteolin, apigenin, acacetin, 3,6,4-trihydroxyflavone, 7,3-dihydroxy-4,1-dimethoxy-isoflavone, 3R-2',3'-
5 dihydroxy-7,4-dimethoxy-isoflavone; or a combination of one or more of the foregoing compounds.

34. The method of Claim 31, wherein treating comprises administering a dosage of about 0.01 mg/kg/day to about 600 mg/kg/day of wogonin.

35. The method of Claim 31, wherein the composition further comprises isoliquiritigenin, coumestrol, or a combination of one or more of the foregoing compounds.

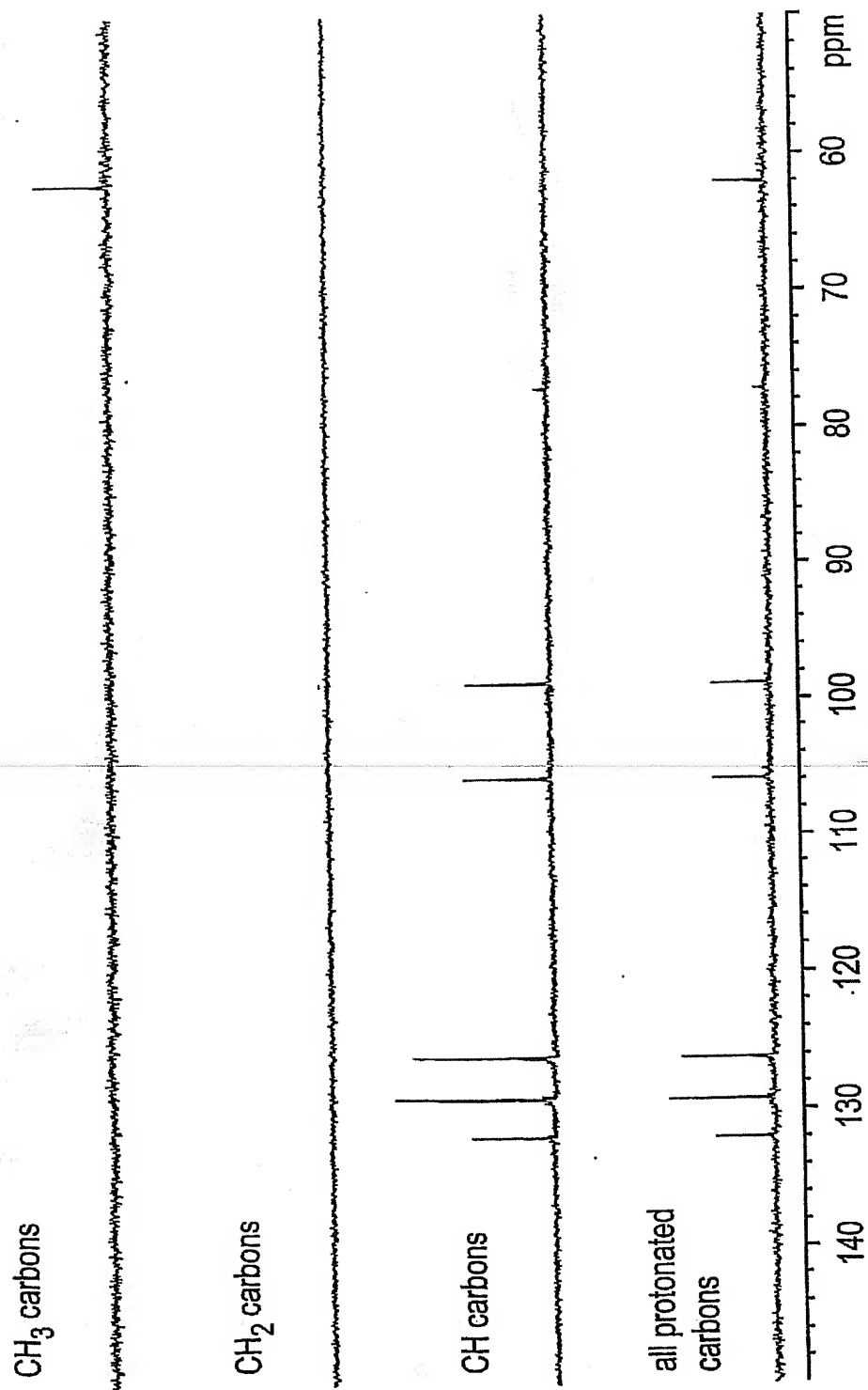
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FIG. 1



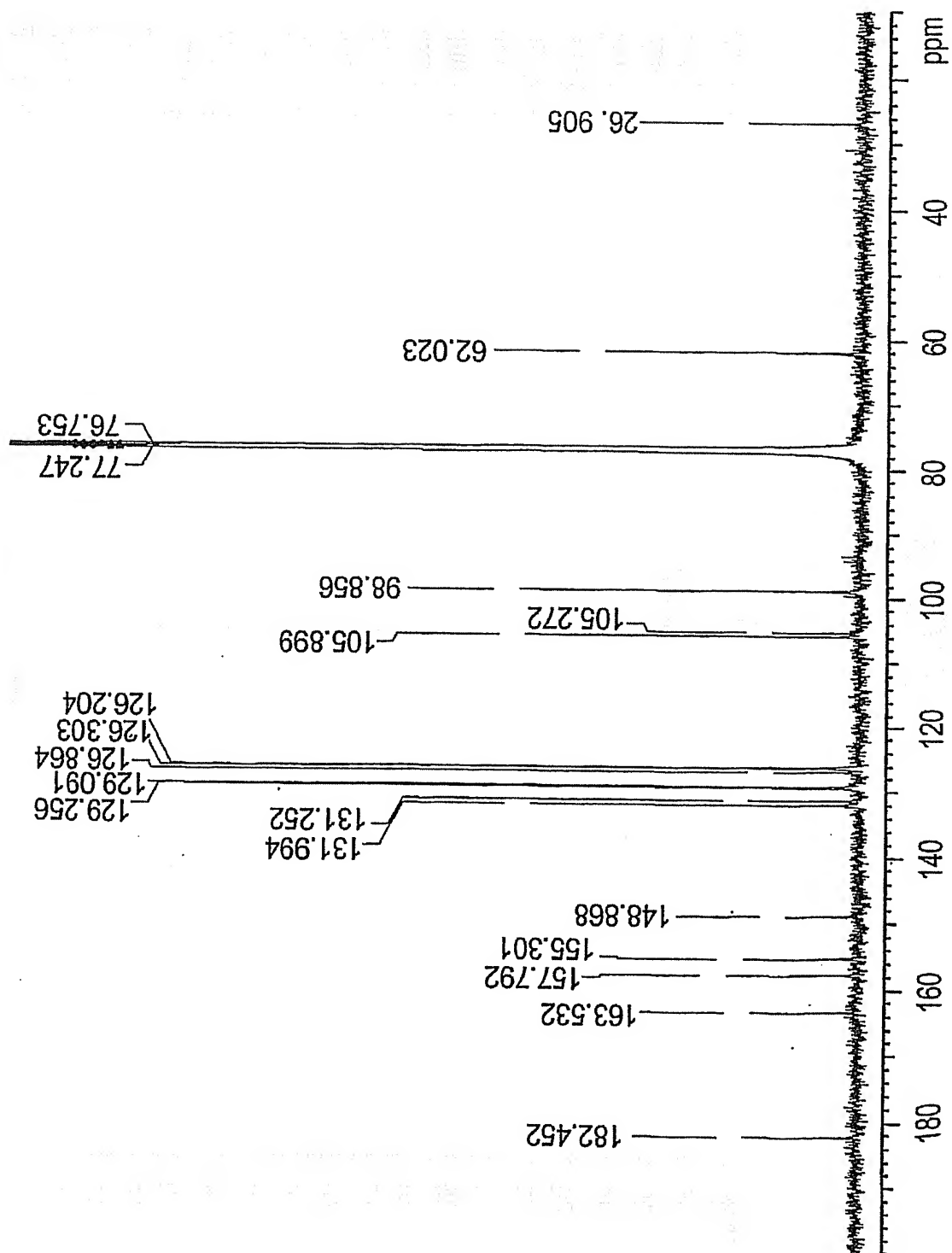
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FIG. 2A



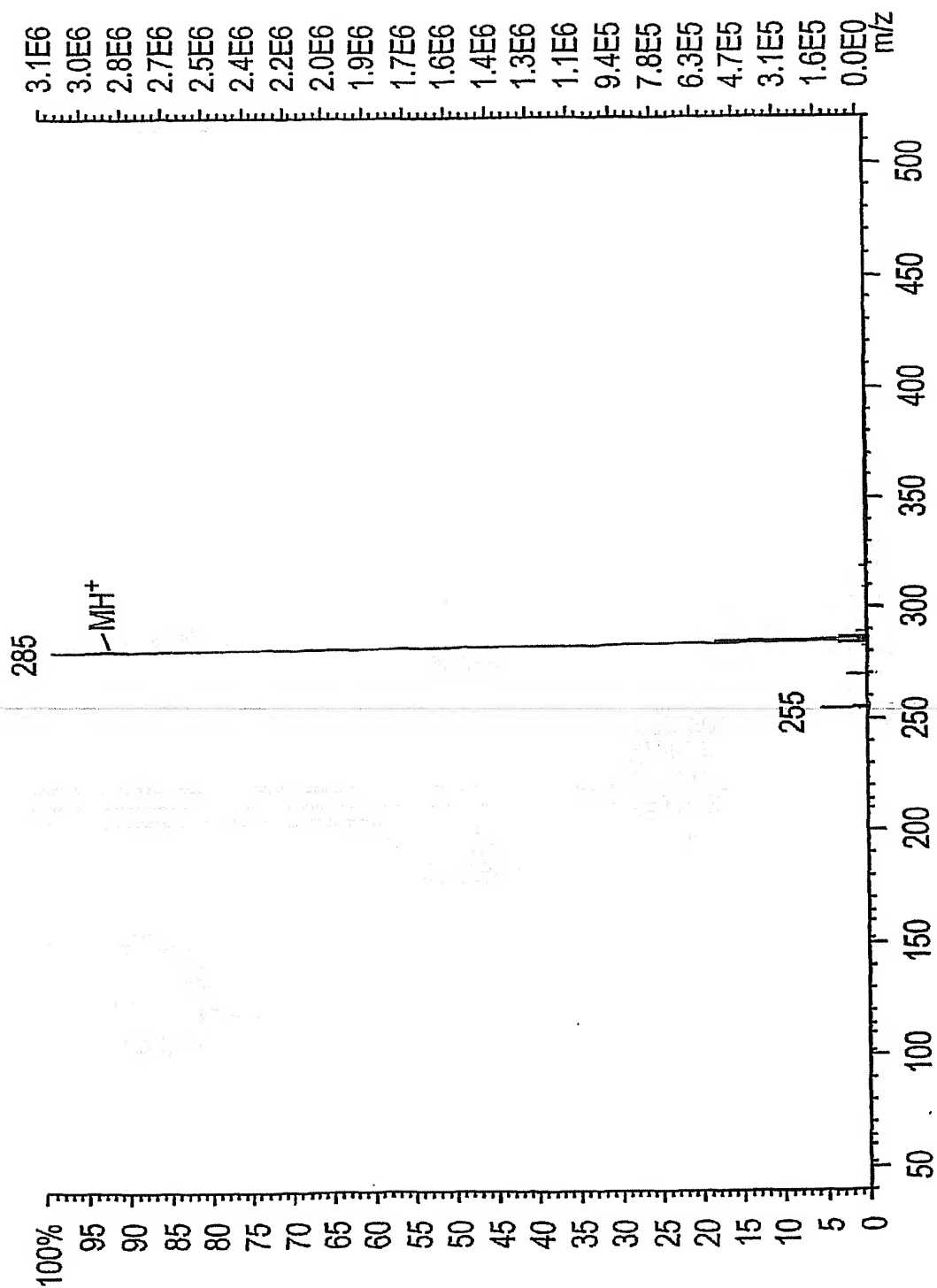
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FIG. 2B



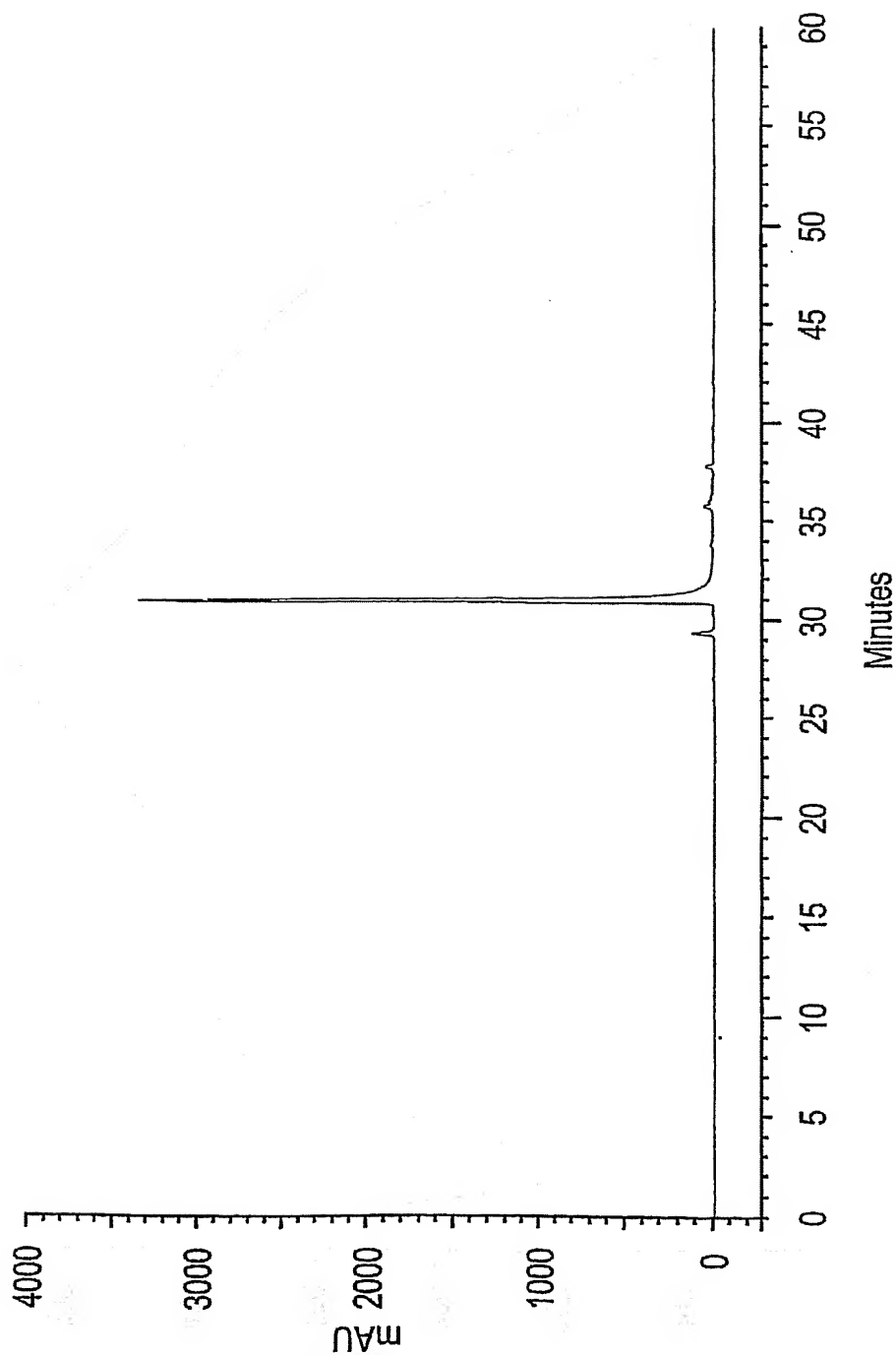
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FIG. 3



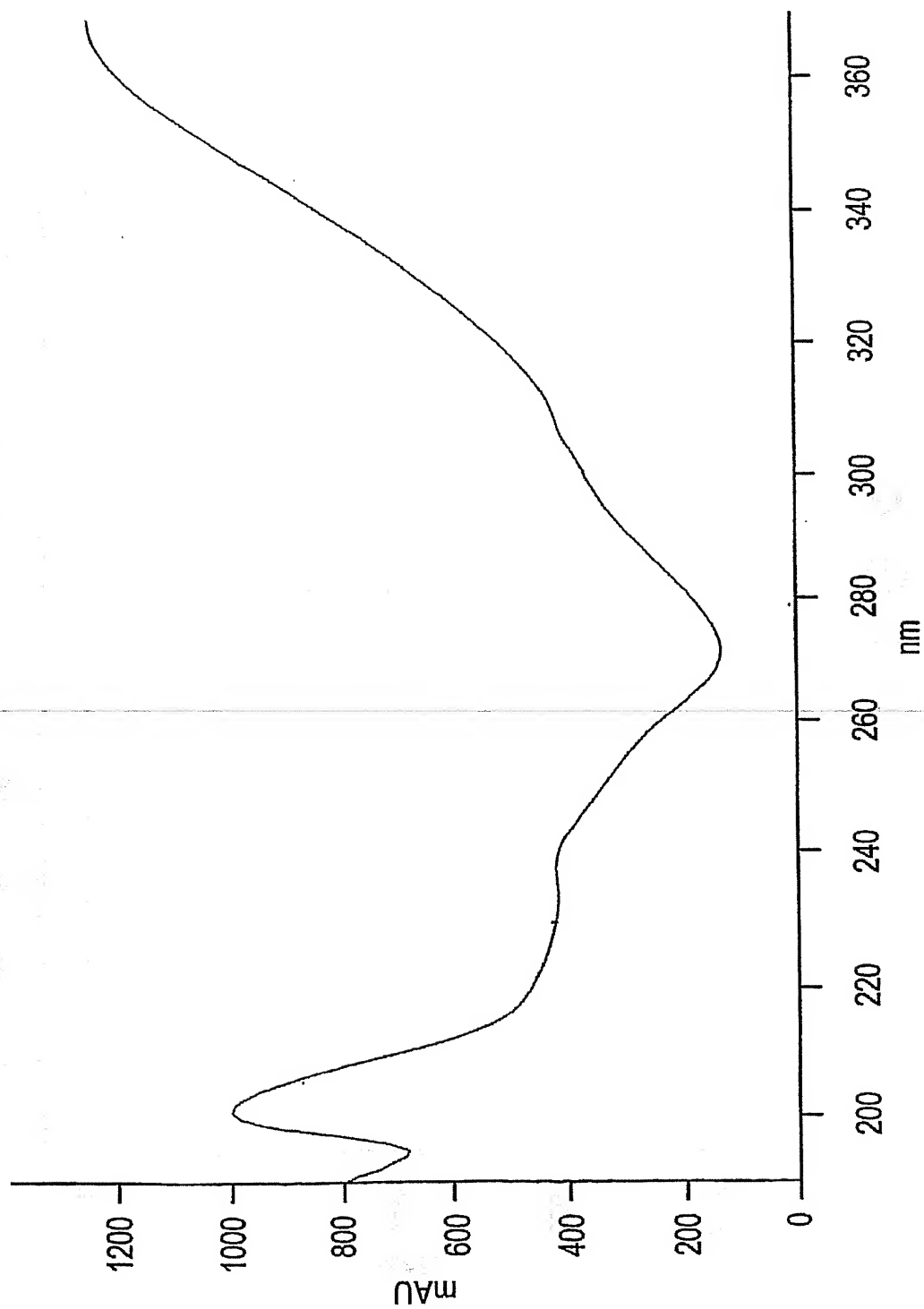
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FIG. 4



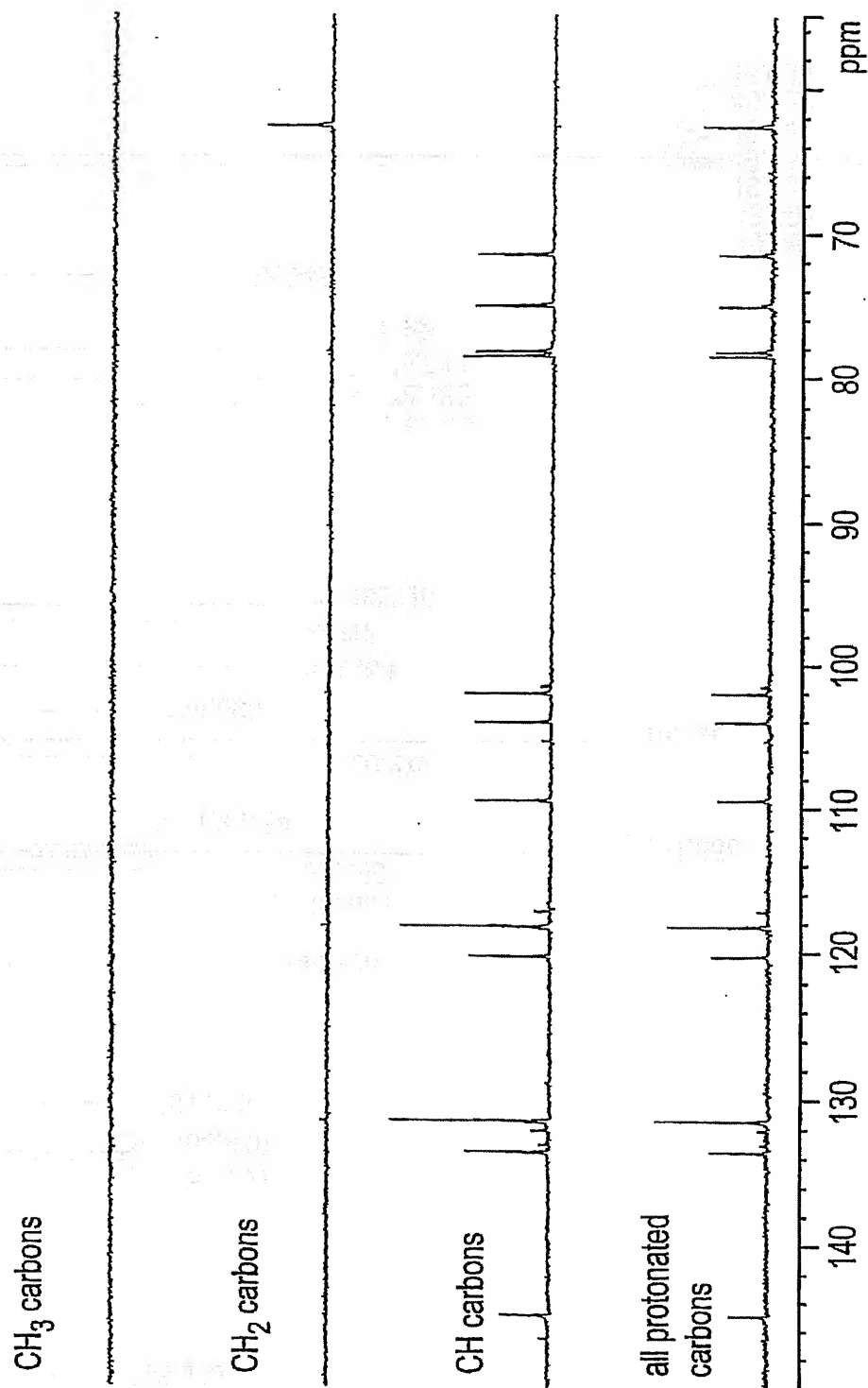
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FIG. 5



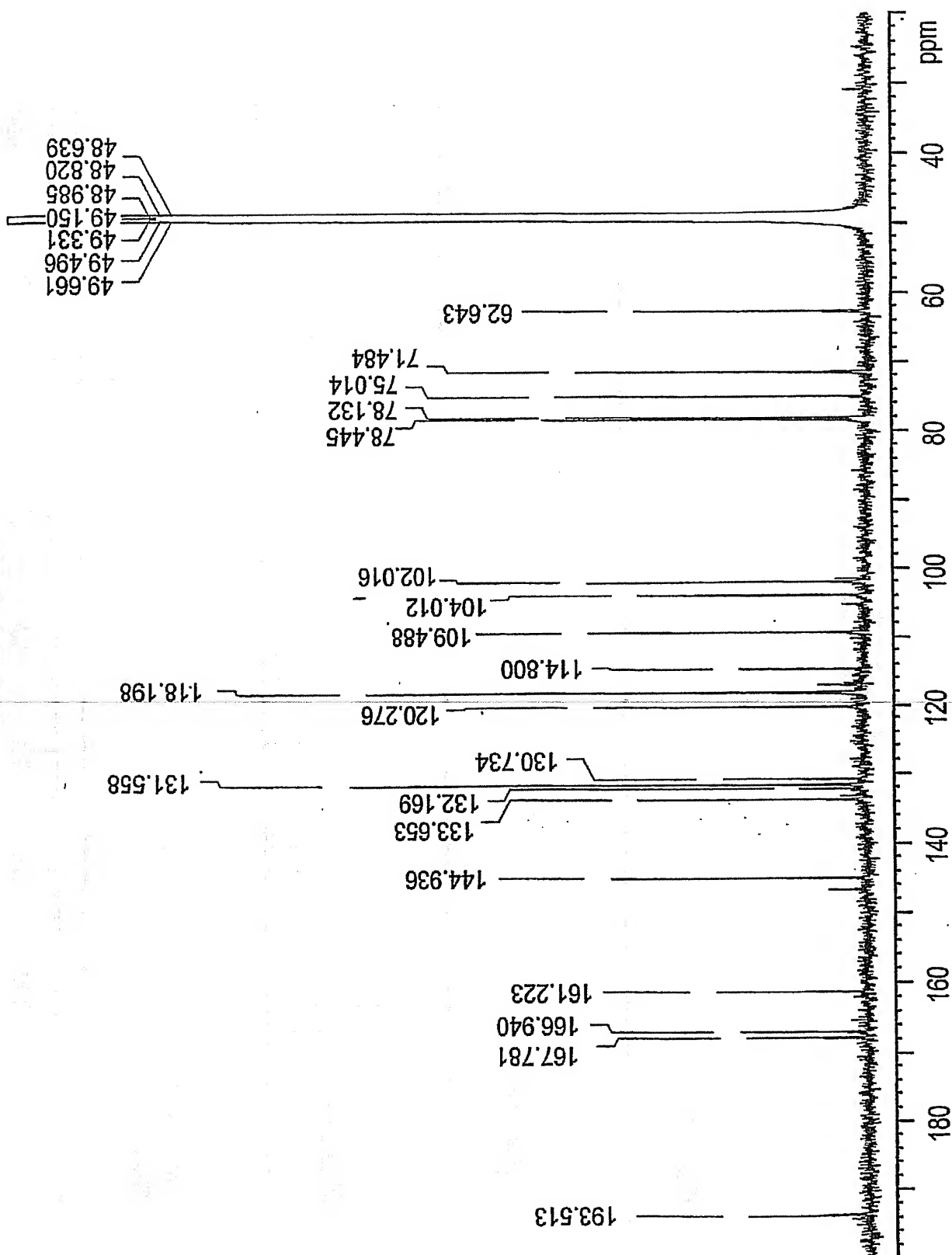
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FIG. 6A



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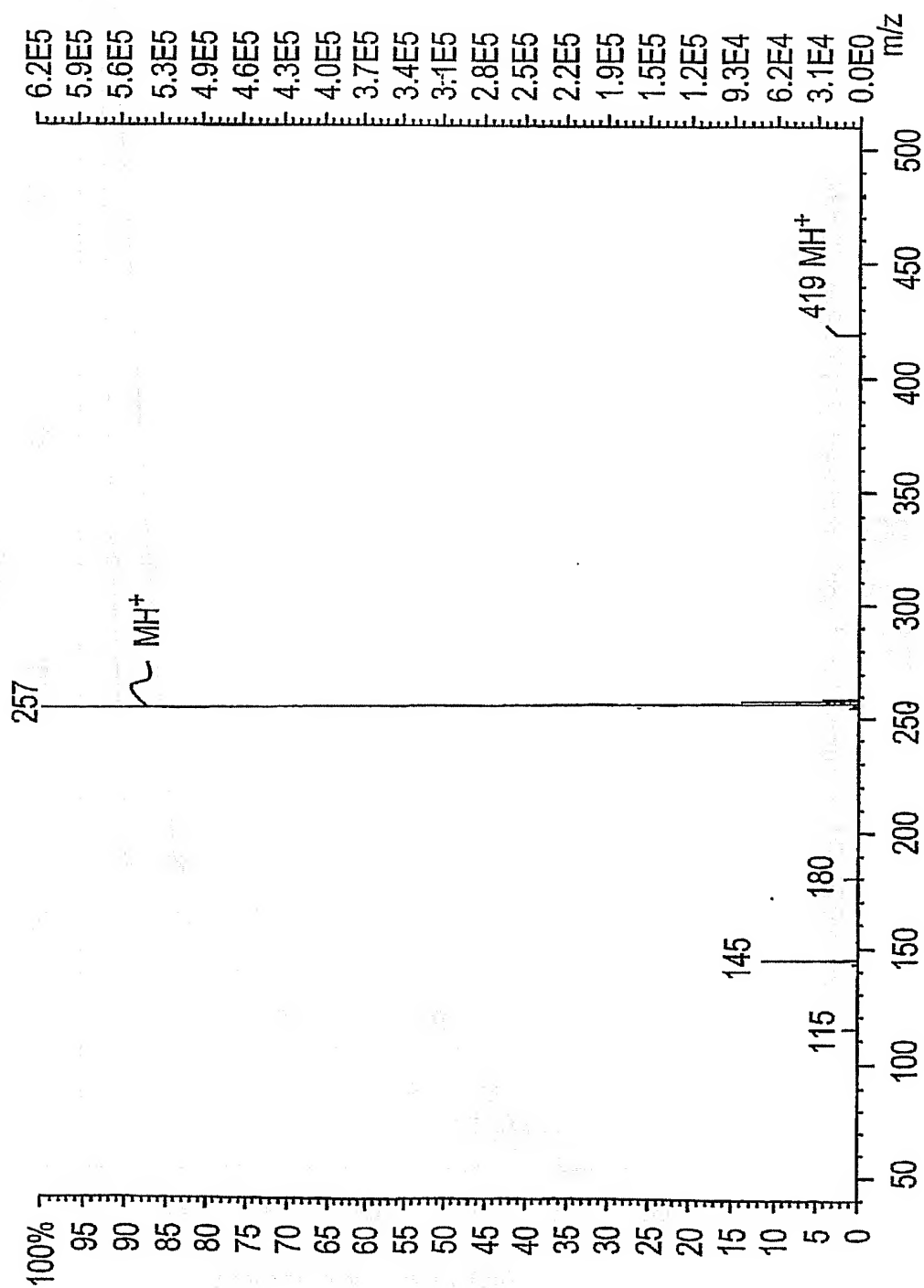
FIG. 6B



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FIG. 7

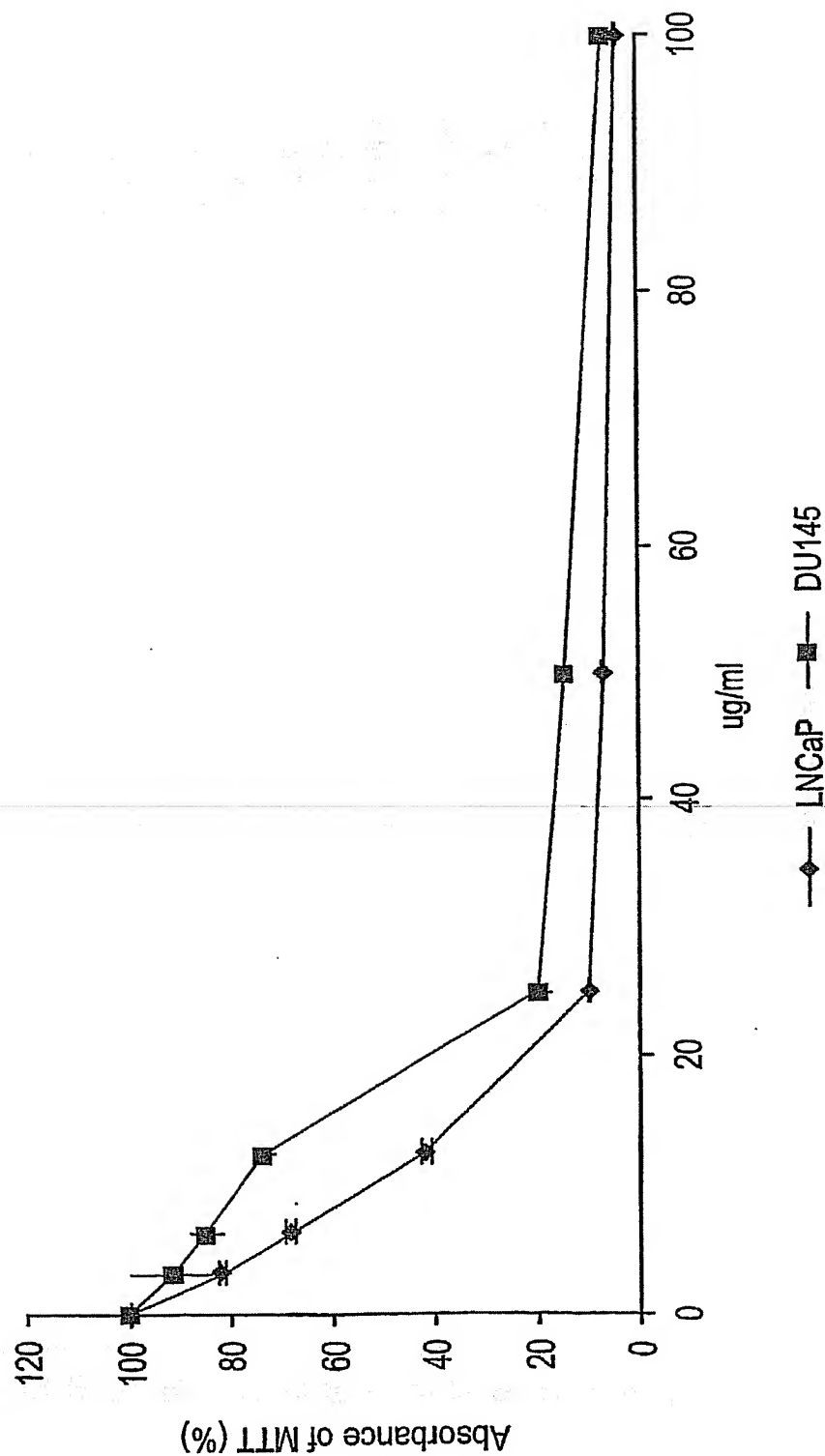
Isoliquiritigenin



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FIG. 8

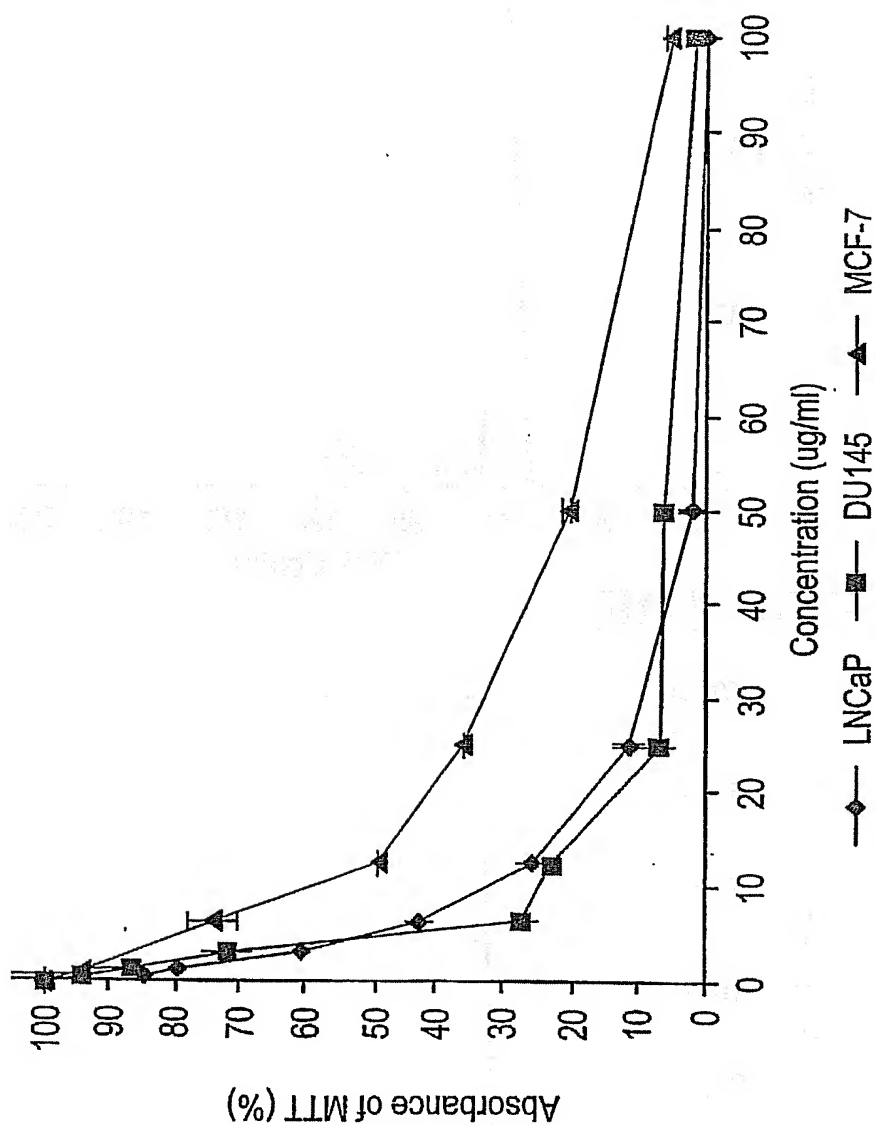
Cell viability (MTT) curves for Wogonin treatment of LNCaP and DU145



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FIG. 9

Cell viability (MTT) curves for Isoliquiritigenin treatment of LNCaP, DU145 and MCF-7



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FIG. 10A

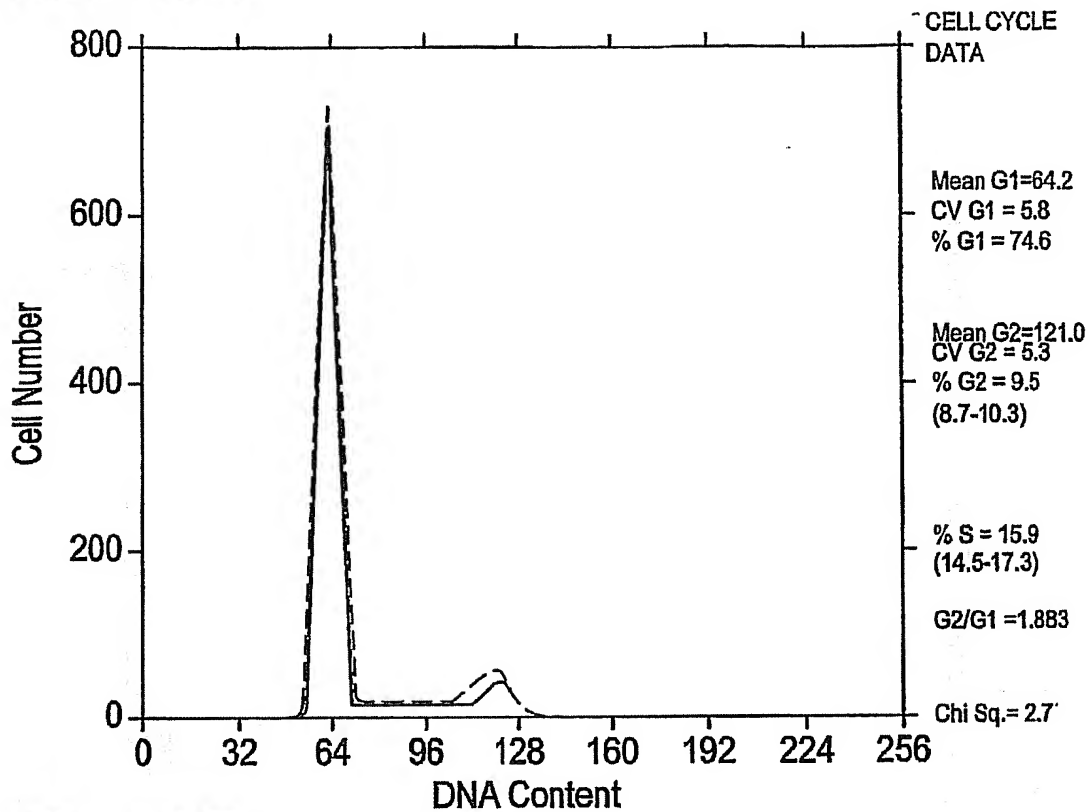
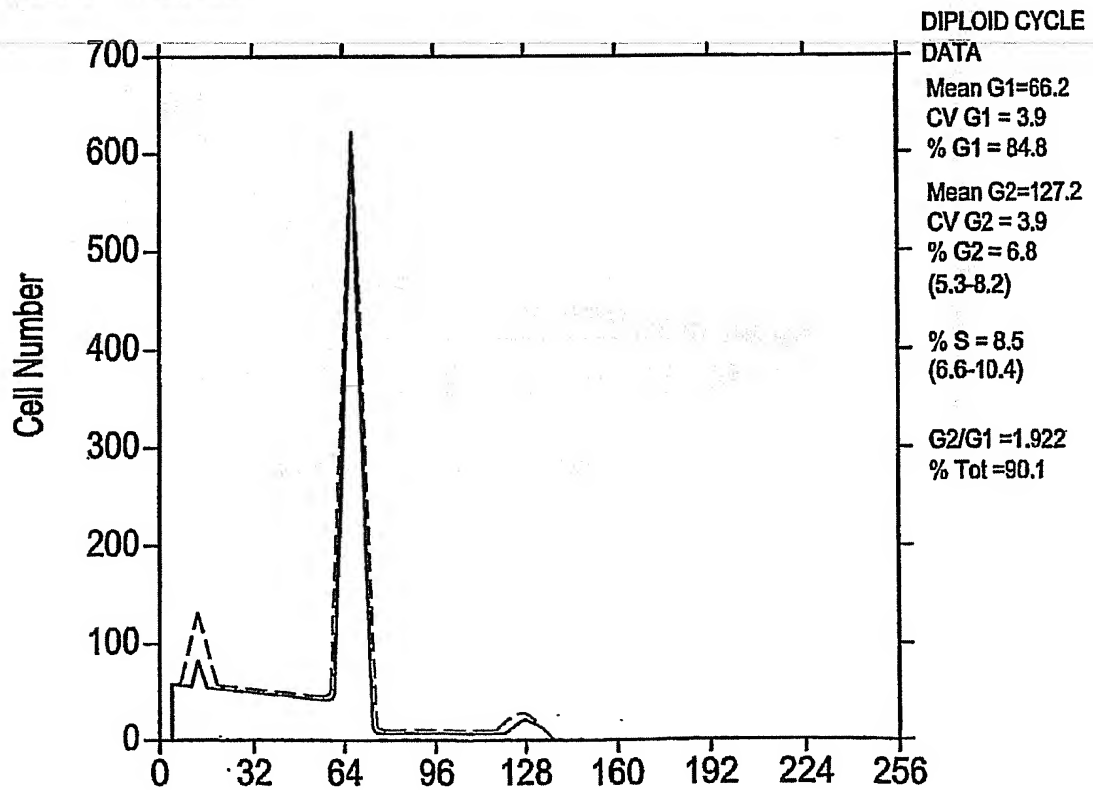
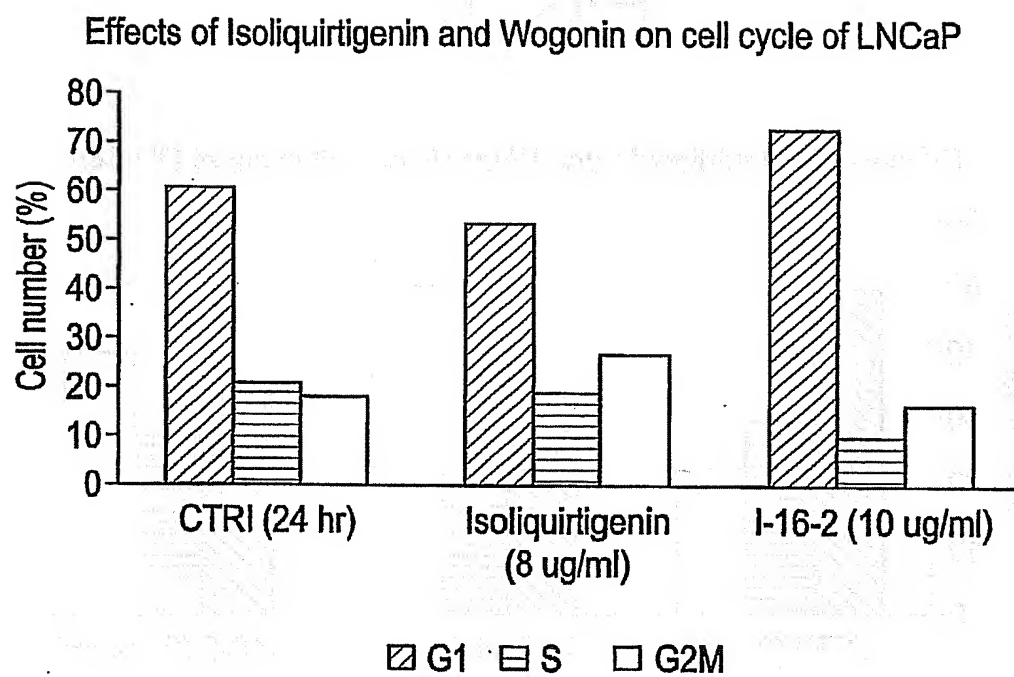


FIG. 10B



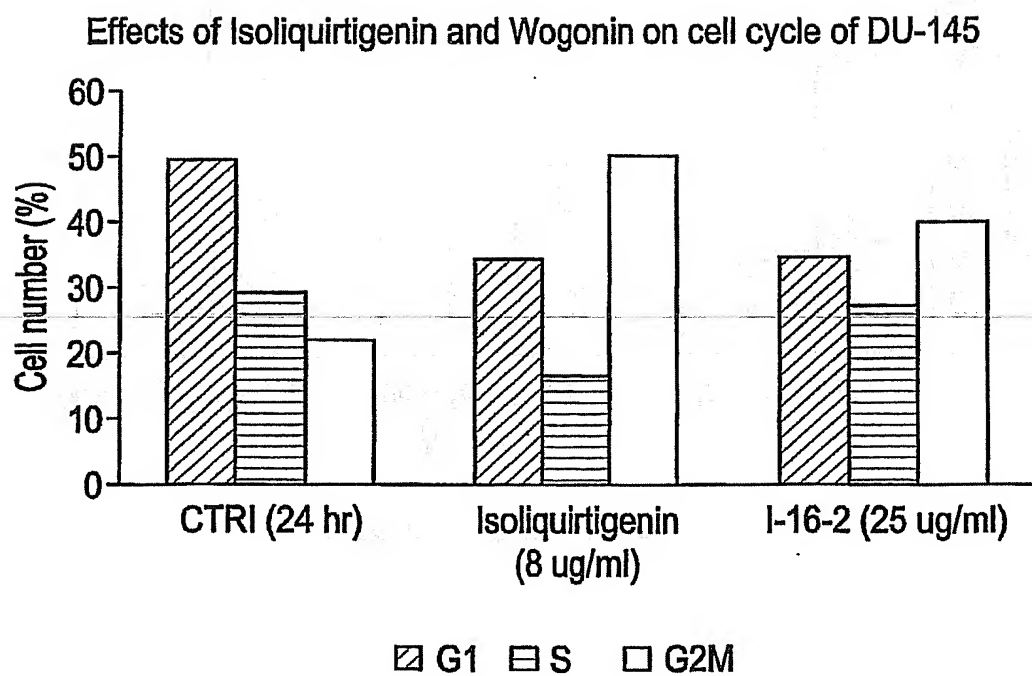
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FIG. 11



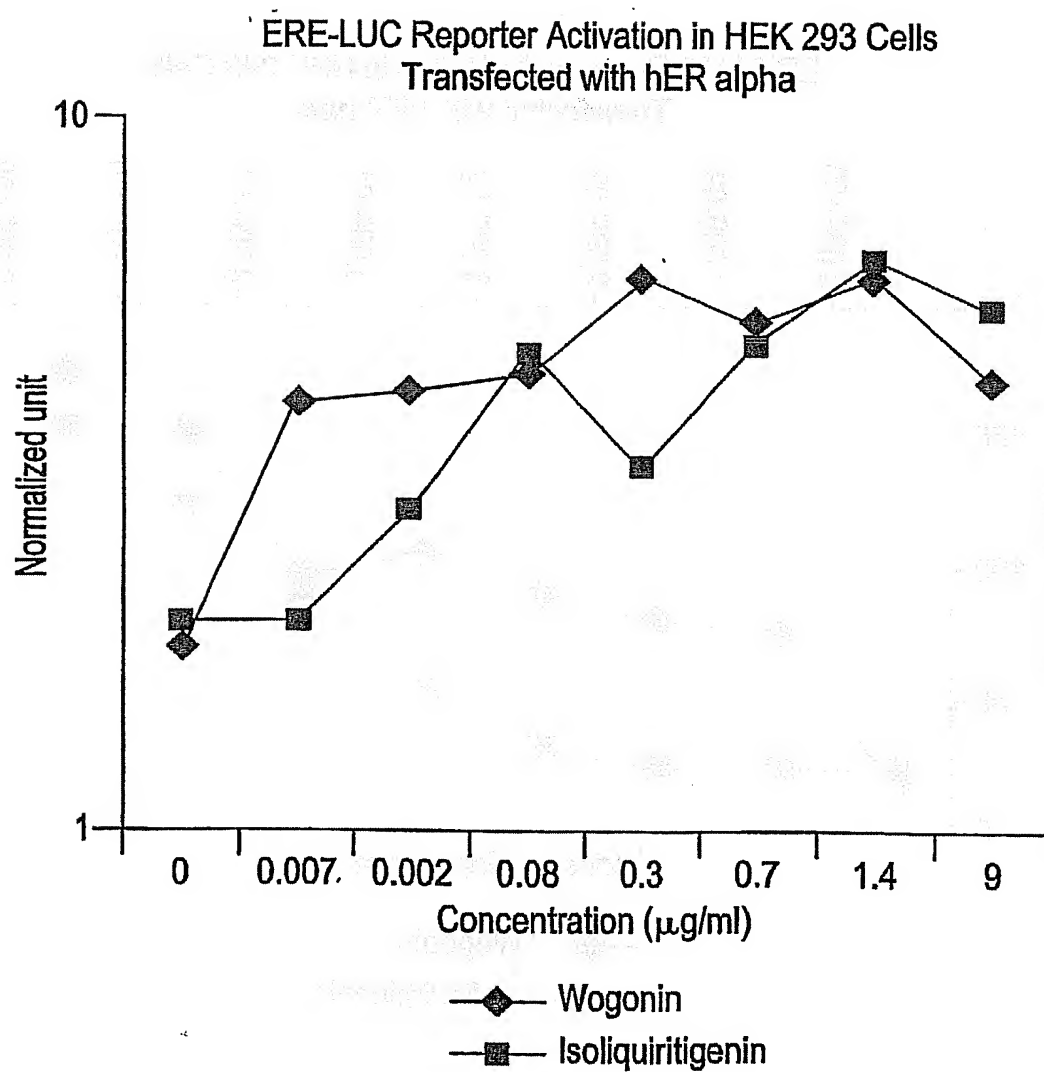
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FIG. 12



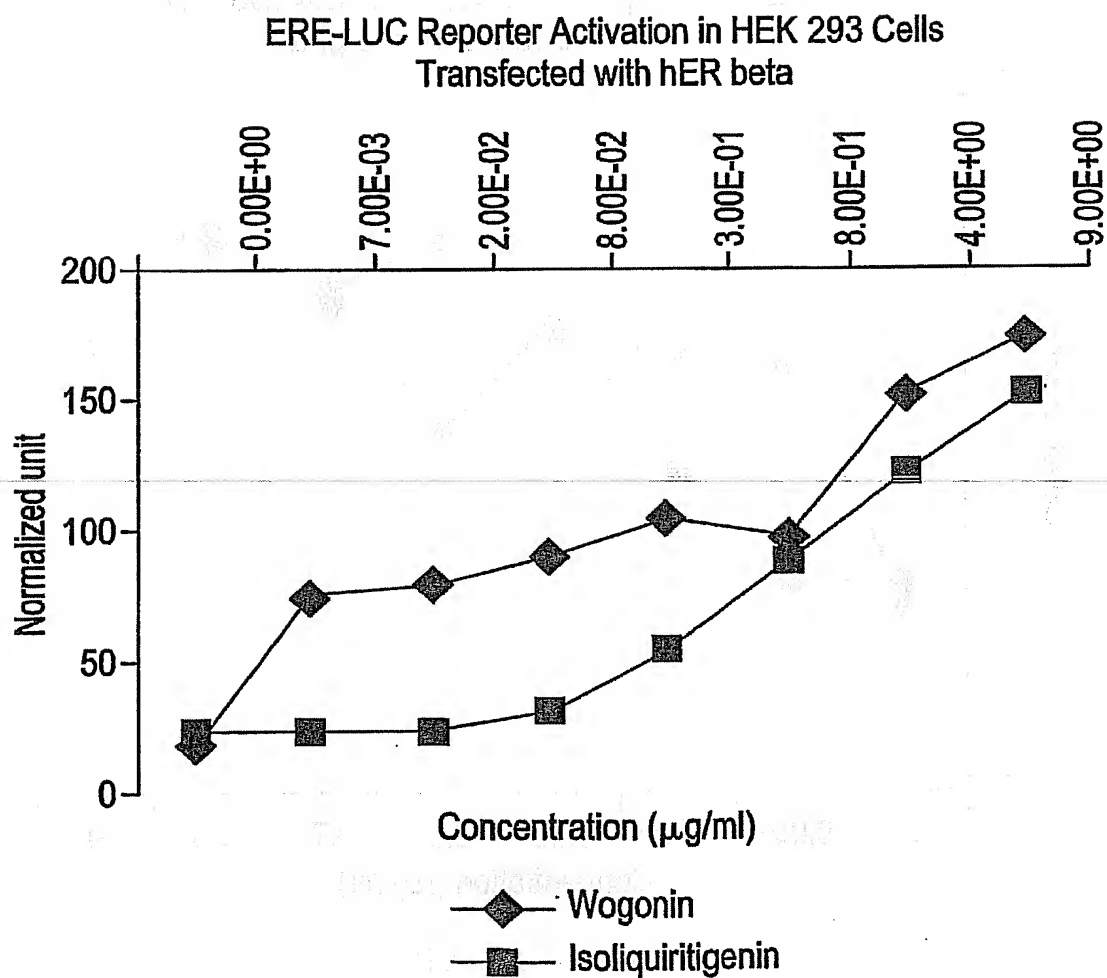
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FIG. 13



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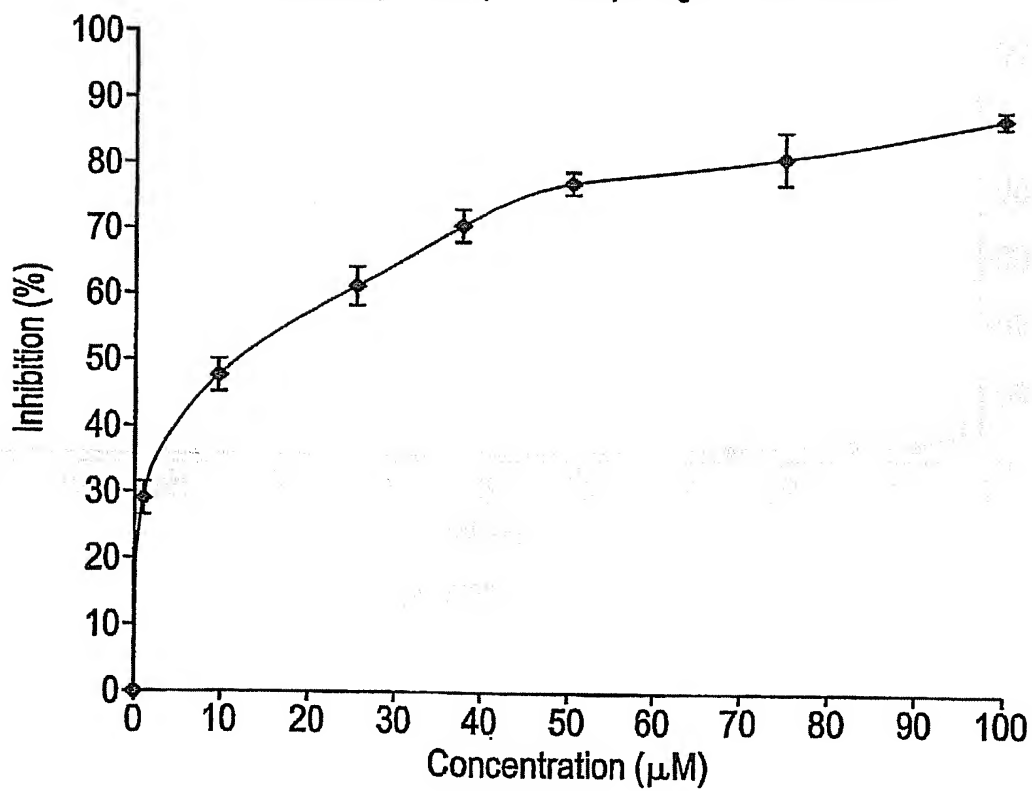
FIG. 14



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FIG. 15

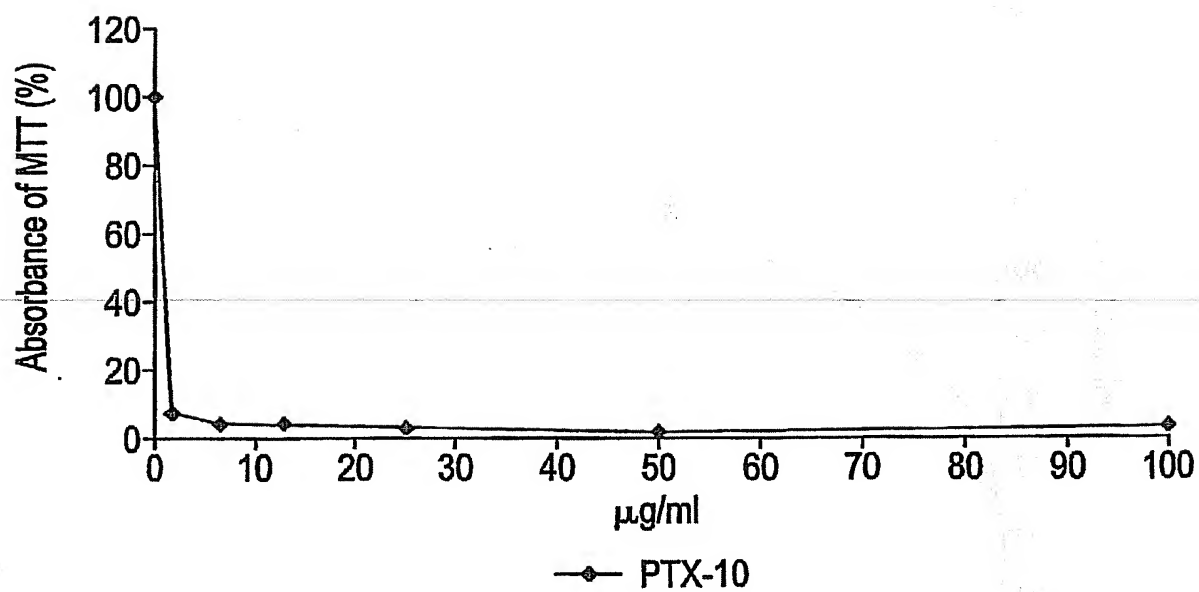
Inhibitory activity of Isoliquiritigenin on COX-2



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FIG. 16

Cell viability (MTT) curves for Wogonin
Treatment of PTX-10



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FIG. 17

Cell viability (MTT) curves for Isoliquiritigenin
Treatment of PTX-10

